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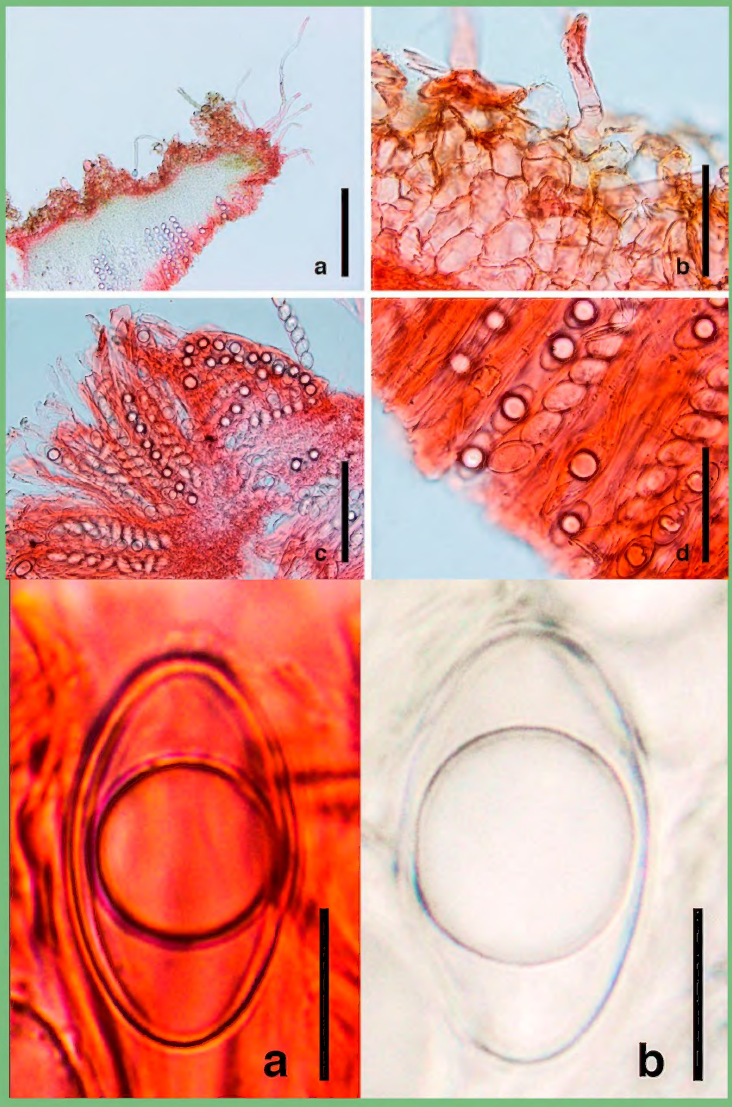

Geopora ahmadii sp. nov.
(Saba & al.— FIGS1 & 2; pp. 382, 383)

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MYCOTAXON

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Reviewers — volume one hundred thirty-four (2)

The Editors express their appreciation to the following individuals who have, prior to acceptance for publication, reviewed one or more of the papers prepared for this issue.

Laise de Holanda Cavalcanti Andrade
Ditte Bandini
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Uwe Braun
Irwin M. Brodo
Rafael F. Castañeda-Ruíz
Maria Martha Dios
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Nadja Santos Vitória
Ping Zhang
Ying Zhang

Errata

Mycotaxon now includes within this section not only errata from past volumes but also corrections of mistakes in the current issue that were present in approved author input files but not detected by authors until after PDF conversion. PDFs will be corrected at deadline for no charge only when they result from editorial error or when authors agree to pay $40 per correction. This section accommodates all other corrections.

Volume 134 (1)

p. 120, line 2–3 — Delete: Mains 1954

Volume 134 (2) [current issue]

p. 225, line 17 — for: "F The nrITS region was amplified …"
read: "The nrITS region was amplified …"

p. 314, line 8 — for: wheat-straw.; read: wheat-straw;

p. 316, line 26 — for: Colonies read: Colonies
(the first letter in 'Colonies' should be in black font)

From the Editor-in-Chief

New to the Editorial Advisory Board—We are pleased to announce the appointment of two new editorial board members. Former Mycotaxon Book Review Editor and University of California researcher, Dr. Else C. Vellinga (who still consents to deliver reviews on a gratifyingly regular basis), is well known to agaricologists through her numerous contributions to the taxonomy of 'lepiotaceous fungi' and as contributing editor to the Flora Agaricina Neerlandica series. Well-schooled in phylogenetic analysis as well as biodiversity and field-work, Else is an active member of the North American Mycoflora Project. Mycologist Dr. Todd Osmundson, Associate Professor of Biology at the University of Wisconsin Lacrosse, also participates in the North American Mycoflora Project. He offers expertise in systematics, biogeography, bioinformatics, DNA barcoding, and molecular phylogenetics and consistently receives high ratings from his students.

The new appointees join Dr. P. Brandon Matheny (University of Tennessee, Knoxville USA) and Dr. Karen Hansen (Curator, Swedish Museum of Natural History (S), Stockholm, Sweden). The newly reconstituted board, which advises Mycotaxon on editorial matters, will elect an two additional members this year.

Elimination of 'MycoBank,' 'IndexFungorum,' 'Fungal Name' preceding identifiers—Members of the Nomenclature for Fungi Committee have discussed the custom of associating numerical fungal identifiers with the name of the assigning database. Because each number is unique, it is not really necessary to designate the issuing nomenclatural registry. Even though your editors and many researchers find such database designations helpful, we agree that it is not necessary to precede an identifier with both name and initials. Mycotaxon has decided to compromise, and henceforth we will cite only the abbreviations FN, IF, and MB, replacing the redundancies Fungal Name FN, IndexFungorum IF, and MycoBank MB. This allows us to focus properly on the number—the formal (and unique) identifier.

Not necessarily "First come, First serve"—Your volunteer two-person editorial team has long tried to review manuscripts in the order in which they are received. Although we view 'fast-tracking' manuscripts as inherently unfair, the Editor-in-Chief has recently begun urging the Nomenclature Editor—our first line of defense against incompetent submissions—to 'cherry-pick' well-prepared work from later submissions to fill out an issue in a timely manner. The situation became even more urgent last year: after we eliminated required page charges, our submissions trebled. For that reason, Shaun, Mycotaxon's primary defensive lineman, announces our revised review policy:

"We attempt to review manuscripts in the order in which they were accessioned. However, in order to meet publication deadlines, badly written and poorly prepared manuscripts that do not follow Mycotaxon's formatting instructions

(and thus require time-consuming editorial reviews) may be set aside in favour of well-presented manuscripts that can be reviewed quickly and easily."

The erratum and how it breeds — We have all experienced the embarrassment of not seeing an error until it pops out on the page of a published manuscript. We all (including this Editor-in-Chief) make mistakes, and it seems only reasonable to expect them to be corrected when reported prior to publication. From 2004–2009 your Editor spent considerable time at the close of each issue reprocessing PDFs when authors 'suddenly' discovered an error that had been in their own text files throughout the entire review process. In 2011, after publication had sometimes been delayed by a week or more while she opened six or more files, corrected mistakes and entries, re-processed PDF proofs, and corresponded with the authors and the press, she announced that we would EITHER publish corrections of errors the authors should have seen in the errata of later volumes for no charge OR correct editorial conversion error prior to publication, also at no charge. Having encountered a number of rather major author errors, this year we initiated a third alternative: authors may pay $40 to have any major author error corrected at press time.

We now offer a fourth alternative: to issue corrections of minor author errors in the same issue in which the paper is published. This option both saves considerable editorial time at deadline and provides the correct text promptly at no charge.

Mycotaxon 134(2) contains 22 papers by 88 authors (representing 10 countries) as revised by 32 expert reviewers and the editors.

The 2019 April–June Mycotaxon proposes 4 new subfamilies—*Lobarioideae, Nephromatoideae, Squamarinoideae, Stereocauloideae*—in the *Lecanoromycetes* plus 15 other taxa new to science representing *Beltraniopsis, Cacumisporium, Cordana, Gliocladiopsis, Megalaria, Pseudocercospora, Seychellomyces, Sporidesmiella,* and *Sporidesmium* from China; *Geopora* and *Ramaria* from Pakistan; *Macrolepiota* from Brazil; and *Perichaena* from Mexico. We also publish the lectotypification of *Lichen squamulosus,* basionym of *Acarospora squamulosa.*

New range extensions reported include *Amoebozoa: Heterostelium* for China; *Ascomycetes:* coprophils for Brazil and *Pseudocercospora* for China; *Basidiomycetes: Inocybe* for Pakistan; and the lichens *Amandinea, Buellia, Haematomma,* and *Heteroplacidium* for China.

Also included are keys to *Beltraniopsis* and *Cacumisporium* species, a list of new GenBank sequences from the newly conserved holotype *Lichen fuscatus* (basionym of *Acarospora fuscata*), and a summary of the new funga covering aphyllophoroid fungi of the Canary Islands, uploaded to the Mycotaxon mycobiota website in May.

Warm regards,
Lorelei L. Norvell (*Editor-in-Chief*)
Shaun R. Pennycook (*Nomenclature Editor*)
28 June 2019

2019 Mycotaxon submission procedure

Prospective Mycotaxon authors should download the Mycotaxon 2019 guide, review & submission forms, and Mycotaxon sample manuscript by clicking the 'file download page' link on our Instructions to Authors page before preparing their manuscript. This page briefly summarizes our '4-step' submission process.

1—Peer review: Authors first contact peer reviewers (two for journal papers; three for mycobiota/fungae) before sending them formatted text & illustration files and the appropriate 2019 Mycotaxon journal or weblist reviewer comment form. Experts return revisions & comments to both the *Editor-in-Chief* <editor@mycotaxon.com> and authors. All co-authors must correct and *proof-read* their files before submitting them to the *Nomenclature Editor*.

2—Nomenclatural review: Authors email all **error-free** text & illustration files to the *Nomenclature Editor* <PennycookS@LandcareResearch.co.nz>. Place **first author surname** + **genus** + 'Mycotaxon' on the subject line, and (required) attach a completed submission form. The Nomenclature Editor will (i) immediately assign the accession number and (ii) after a few weeks return his notes and suggested revisions to the author(s) and *Editor-in-Chief*.

3—Final submission: All coauthors thoroughly revise and proof-read files to prepare error-free text and images ready for immediate publication. Poorly formatted copy will be rejected or returned for revision. They email the final manuscript to the *Editor-in-Chief* <editor@mycotaxon.com>, adding the **accession number** to the message and **all** files, which include a **(i)** revised 2019 submission form, all **(ii)** text files and **(iii)** jpg images, and **(iv)** FN, IF, or MB identifier verifications for each new name or typification. The *Editor-in-Chief* acknowledges submissions within two weeks of final submission but requests authors to wait at least 14 days before sending a follow-up query (without attachments).

4—Final editorial review & publication: The *Editor-in-Chief* conducts a final grammatical and scientific review and returns her editorial revisions to all expert reviewers and coauthors for final author approval. Author-approved files are placed in the publication queue.

The PDF proof and bibliographic & nomenclatural index entries are sent to all coauthors for final inspection. After PDF processing, the *Editor-in-Chief* corrects only PDF editorial/conversion and index entry errors; corrections of all other errors are listed in the Errata of a subsequent issue for no charge. Authors will pay fees for mycobiota uploads, optional open access, and correction of major author errors to the *Business Manager* <subscriptions@mycotaxon.com> at this time.

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Volvate *Macrolepiota* from Brazil: *M. dunensis* sp. nov., *M. sabulosa* var. *velistellaris* var. nov., and observations on *M. pulchella*

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Abstract—Taxonomic and phylogenetic studies of volvate *Macrolepiota* specimens collected from Atlantic Forest in Northeast and Southeast Brazil are presented based on morphological and molecular (nrITS) data. *Macrolepiota dunensis* and *M. sabulosa* var. *velistellaris* are proposed as new taxa, and additional observations are presented about *M. pulchella*, a species previously known from Brazil. *Macrolepiota dunensis* and *M. pulchella* clustered in the clade corresponding to *M.* sect. *Volvatae*. *Macrolepiota sabulosa* var. *velistellaris* was placed together with non-volvate *M. sabulosa* var. *sabulosa*, close to another volvate taxon (*M. rhodosperma* var. *velicopia*), and together with species classified in *M.* sect. *Macrolepiota*. Our phylogenetic results provide additional evidence that the volva, which is not restricted to members of *M.* sect. *Volvatae*, evolved more than once in the genus.

Key words—*Agaricaceae*, *Lepiotella*, Neotropics, South America, *Volvolepiota*

Introduction

Macrolepiota Singer (*Agaricaceae*) was proposed by Singer (1948) to accommodate specimens characterized by giant basidiospores that are metachromatic in Cresyl blue, a white to cream spore print, and hyphae with clamp connections. Singer (1959) proposed *Volvolepiota* Singer for the

illegitimate genus *Lepiotella* Rick (Rick 1938), separated from *Macrolepiota* by the presence of a distinct volva. At present morphological and molecular data (Vellinga 2003; Vellinga & al. 2003) treat *Macrolepiota* and *Volvolepiota* as synonyms, with *Macrolepiota* restricted to specimens that in addition to Singer's diagnostic characters (1948, 1986) possess a trichodermal pileus covering composed of long and often thick-walled elements, a stipe covered with hymeni-trichodermal patches often arrayed in colored bands, and basidiospores with a rounded apex and a germ pore covered by a hyaline cap. The current concept of *Macrolepiota* also includes sequestrate species (Lebel & Syme 2012). Supported by nrITS sequence analyses, Ge & al. (2010) proposed *Macrolepiota* sect. *Volvatae* Z.W. Ge & al. to accommodate volvate species with small amygdaliform-ellipsoid basidiospores and no clamp connections.

Morphological and molecular studies support the existence of only five volvate *Macrolepiota,* distributed mainly in tropical regions: *M. brunnescens* Vellinga from Argentina (Vellinga & Yang 2003; Singer 1959 as *Volvolepiota albida*); *M. eucharis* Vellinga & Halling from Australia (Vellinga 2003); *M. pulchella* from Brazil (Vellinga & Yang 2003, de Meijer 2006; Rick 1938, 1961, as *Lepiotella brunnea*; Singer 1954, 1959, Heinemann & de Meijer 1996, Bononi & al. 1981, as *V. brunnea*); *M. rhodosperma* var. *velicopia* Vizzini & Contu from Italy (Vizzini & al. 2011); and *M. velosa* Vellinga & Zhu L. Yang from China (Vellinga & Yang 2003).

Ge & al. (2010), who included the volvate species *Macrolepiota velosa* and *M. eucharis* in *M.* sect. *Volvatae,* suggested that *M. brunnescens* and *M. pulchella* might also belong to this section. The description of a volvate taxon (*M. rhodosperma* var. *velicopia*) not phylogenetically related to *M.* sect. *Volvatae* representatives (Vizzini & al. 2011) provoked a new discussion about recognizing a natural classification of *M.* sect. *Volvatae* based on velar structures.

The only volvate *Macrolepiota* recorded from Brazil is *M. pulchella*, known from the southern and southeastern states of Rio Grande do Sul (Rick 1938, 1961, as *L. brunnea*; Singer 1954, 1959, as *V. brunnea*), São Paulo (Bononi & al. 1981, as *V. brunnea*), and Paraná (de Meijer 2006; Heinemann & de Meijer 1996, as *V. brunnea*).

Here we report morphological and molecular data for three volvate *Macrolepiota* taxa based on specimens collected in the Atlantic Forest of Rio Grande do Norte (Northeast Brazil) and São Paulo (Southeast Brazil). Two—*M. dunensis* and *M. sabulosa* var. *velistellaris*—are described as new, and we discuss the taxonomy and phylogenetic position of the third, *M. pulchella*.

Materials & methods

Morphological studies

The macroscopic and ecological descriptions are based upon collector's notes from fresh materials. Color terms follow Küppers (1979). For microscopic analyses, sections of lamellae, pileus covering, and stipe were moistened in 70% ethanol and rehydrated in 5% KOH. Reagents used were Congo red to stain hyaline structures, Cresyl blue to test metachromasy, and Melzer's reagent to test basidiospore amyloidy. Microscopic illustrations were made with the aid of a drawing tube attached to an Olympus BX50 compound microscope. For basidiospore measurements, [a/b/c] indicates "a" basidiospores measured from "b" basidioma(ta) taken from "c" collection(s); Q = length/width quotients from all measured basidiospores, Qm = average of all computed Q values; Lm (Wm) = average of lengths (widths) of all measured basidiospores. At least 20 basidiospores from each basidioma were measured in profile view. Basidiospore shape terminology follows Bas (1969), and descriptive terms for other morphological features follow Vellinga (1988) and Vellinga & Noordeloos (2001). The terminology of vegetation types is according to Veloso & al. (1991), and the herbarium acronyms follow Thiers (2018).

Molecular methods and phylogenetic analyses

DNA extraction, PCR, and sequencing methods followed Justo & al. (2011a,b). F The nrITS region was amplified and sequenced using the primer pair ITS-1 and ITS4 (White & al. 1990; Gardes & Bruns 1993). The newly generated sequences were phylogenetically analysed with other DNA sequences of the main representatives of *Macrolepiota* available in GenBank and *Leucoagaricus leucothites* (Vittad.) Wasser and *L. nympharum* (Kalchbr.) Bon as outgroup. GenBank accession numbers and other collection information for the sequences used in molecular analyses are given in Fig. 1. Our alignment matrix, generated by MAFFT 7 (http://mafft.cbrc.jp/alignment/server/; Katoh & Toh 2008) with auto strategy, was visually examined and manually corrected using Geneious 7.0.6 (Kearse & al. 2012).

To identify the best nucleotide evolution model, we used the AICc criterion – Akaike Information Criterion corrected (Hurvich & Tsai 1991) in PartitionFinder 2.1.1 (Lanfear & al. 2017). After separating the ITS region into ITS1+ITS2 and 5.8S, we estimated the evolution model for each partition. Maximum Likelihood (ML) analysis was performed in RAxML v8.2.X (Stamatakis 2014) using the GTR+G model with a rapid bootstrap analysis with 1000 replicates and search for the best-scoring ML tree. The Bayesian inference (BI) analysis was performed with MrBayes v3.2.6 (Ronquist & al. 2012) in the CIPRES Science Gateway 3.1 (Miller & al. 2010) using two partitions with the TVM+G model for ITS1+ITS2 and the JC model for 5.8S, with two independent runs, four simultaneous independent chains, and 10,000,000 generations with a sample frequency every 5000 generation. The following abbreviations are used for statistical data: Bootstrap (BS) and Posterior Probability (PP).

Results & discussion

We would prefer to sample additional collections with a broader morphological variation to support our new taxa and to provide more information about the ontogeny and distribution. Nonetheless, because only five volvate *Macrolepiota* are currently known and because morphological and molecular characters support our material as different taxa, we propose new taxa here to advance *Macrolepiota* research: *M. dunensis* as a new species and *M. sabulosa* var. *velistellaris* as a new variety that distinguished from *M. sabulosa* var. *sabulosa* mainly by the presence of a distinct volva on the stipe base.

Phylogeny

Both ML and BI analyses established the phylogenetic positions of the three Brazilian volvate *Macrolepiota* taxa. The ML tree (Fig. 1) organized the *Macrolepiota* species in three main clades corresponding to the sections indicated by Ge & al. (2010)—*M.* sect. *Macrolepiota*, *M.* sect. *Macrosporae* (Singer) Bon, and *M.* sect. *Volvatae*.

The clade corresponding to *Macrolepiota* sect. *Volvatae* includes sequences from *M. dunensis*, *M. eucharis*, *M. pulchella*, *M. velosa*, and other unidentified collections from Brazil and related to *M. pulchella*. All identified specimens in this clade possess a distinct volva on the stipe base. *Macrolepiota pulchella* clustered with other three sequences of unidentified specimens from Brazil in a well-supported (100% BS, 1 PP) clade (Fazolino Perez & al. 2018), with *M. dunensis* and *M. eucharis* on an external branch. Although we still need to review the morphology of the unidentified collections reported by Fazolino Perez & al (2018) as *Macrolepiota* sp. 3, our molecular results indicate that they are most likely *M. pulchella*. The level of sequence divergence among *M. pulchella* and *Macrolepiota* sp. 3 is up to 1% and is represented by three different base pairs, with an additional different base pair in *Macrolepiota* sp. 3 KY927723 and four ambiguous base pairs in *M. pulchella* MC4411.

The other new volvate *Macrolepiota* (*M. sabulosa* var. *velistellaris*) clusters in the *M.* sect. *Macrolepiota* clade with sequences of the non-volvate typical variety of *M. sabulosa* Fazolino & R.M. Silveira from Brazil (Fazolino Perez & al. 2018), and sequences of *M. cyanolamellata* Fazolino & al. from Brazil (Fazolino Perez & al. 2018) and *M. clelandii* Grgur. from Australia and New Zealand (Vellinga 2003, Vellinga & al. 2003). Although the sequence from *M. sabulosa* var. *velistellaris* is 100% identical to that from the holotype of *M. sabulosa* (and its autonymous variety *M. sabulosa* var. *sabulosa*)—

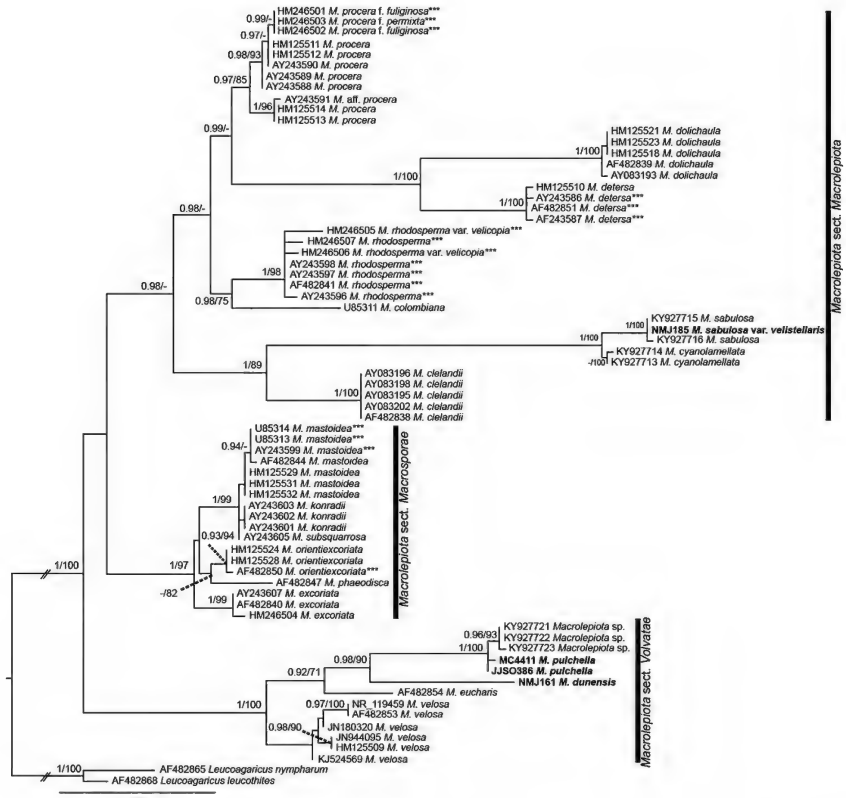

FIG. 1. Bayesian Inference tree of *Macrolepiota* based on nrITS data and rooted with *Leucoagaricus leucothites* and *L. nympharum*. The sequences generated in this work are in bold. Support values (PP >0.90 and BS >70%) are shown above the node branches. *** indicates taxon names modified according to Vizzini & al. (2011).

KY927715, we prefer to propose a new variety based on the morphological differences discussed below.

Vizzini & al. (2011) described another volvate *Macrolepiota* taxon, *M. rhodosperma* var. *velicopia*, which differs from the autonymous variety, *M. rhodosperma* var. *rhodosperma*, primarily by the presence of a volva on the stipe base. *Macrolepiota rhodosperma* var. *velicopia,* which clusters in the *M.* sect. *Macrolepiota* clade, is not phylogenetically related to *M.* sect. *Volvatae*. Vizzini & al. (2011) noted that the presence of a volva on the stipe base is a character that developed independently over the evolution of

Macrolepiota species, suggesting that recognition of *M.* sect. *Volvatae* based solely on the presence of volva is an artificial classification. Additionally, Ge & al. (2010) highlighted the relatively small (usually <15 µm) amygdaliform-ellipsoid basidiospores and absence of clamp connections as additionally diagnostic of *M.* sect. *Volvatae*. The position of *M. sabulosa* var. *velistellaris* outside *M.* sect. *Volvatae* supports the view of Vizzini & al. (2011) regarding the homoplasy of the volva within *Macrolepiota*.

Our sequence analyses also separate *Macrolepiota dunensis* and *M. sabulosa* var. *velistellaris* from all known and sequenced volvate *Macrolepiota* taxa and present for the first time molecular data for *M. pulchella*, the only volvate *Macrolepiota* species previously recorded from Brazil. It is important to note that our phylogeny was generated only from nrITS data and that the internal position and the actual relationships of the sampled taxa may differ when more markers are used.

Taxonomy

Macrolepiota dunensis D.S. Freitas & Menolli, **sp. nov.** FIGS 2A,D, 3

MB823088

Differs from *Macrolepiota pulchella* by its shorter stipe, its lack of triangular patches or squamules on the pileus disc, its smooth and non-encrusted cheilocystidia, and its shorter terminal elements in the pileus covering.

TYPE—Brazil. Rio Grande do Norte: Natal, Parque Estadual Dunas de Natal, Geology trail, 12 July 2010, Menolli Jr. & al. NMJ161 (**Holotype**, SP466944; GenBank MG136892).

ETYMOLOGY—*dunensis* is the Latinized form of dune, for the sand dune area in an Atlantic Forest fragment where the holotype was collected.

PILEUS 50 mm diam., plano-concave, with an obtuse umbo, dark brown ($N_{80}A_{30}M_{50}$) at center, fully covered with appressed fibrils up to two-thirds of the radius, slightly lighter ($N_{80}A_{50}M_{50}$) elsewhere and with the innate fibrils dissociating radially on cream background. LAMELLAE free, ventricose, crowded, cream colored, with lamellulae. STIPE 55 × 3(apex)/7(base) mm, central, brown ($N_{70}A_{60}M_{50}$) and slightly lighter at the apex, covered by inconspicuous zigzag bands mainly on central part, tapering upwards and enlarged at base, fibrous and slightly striate longitudinally. ANNULUS lost but with clear mark on the central stipe. VOLVA limbate, membranous with a cottony base, dirty white to light brown at the margin ($N_{40}A_{30}M_{20}$). CONTEXT, ODOR, TASTE, AND SPORE PRINT COLOR not recorded.

FIG. 2. A, D. ***Macrolepiota dunensis*** (holotype, SP466944). B, E. ***Macrolepiota pulchella*** (B = SP466949; E = SP381606). C, F. ***Macrolepiota sabulosa* var. *velistellaris*** (holotype, SP466945). Scale bars = 1 cm. Photos: A, D = N. Menolli Jr.; B = J.J.S. Oliveira; C, F = I.G. Baseia; E = M. Capelari.

BASIDIOSPORES [25/1/1] 9.0–12.0 × 6.0–8.0 µm (Q = 1.4–1.8; Qm = 1.6; Lm = 10.7 µm; Wm = 6.8 µm), ellipsoid to elongate, smooth, thick-walled, hyaline, with an apical central truncate germ pore covered by a hyaline cap, some with an inconspicuous apiculus, dextrinoid in Melzer's and metachromatic in Cresyl blue. BASIDIA 26–31 × 11.2–13.0 µm, clavate, hyaline, thin-walled to slightly thickened at the apex, 2-, 3-, and 4-spored in equal abundance. CHEILOCYSTIDIA 32–47 × 7.5–11.2 µm, cylindrical to narrowly clavate, hyaline, thin-walled to slightly thickened towards the apex. HYMENOPHORAL TRAMA irregular composed of thin-walled hyphae, 10.0–16.2 µm diam., hyaline. PILEUS TRAMA hyphae thin-walled, 8.7–16.2 µm diam., with light brown content. PILEUS COVERING an intricate trichoderm, composed of cylindrical elements with light brown content, thin-walled, terminal elements 77–171 × 5.0–7.5 µm. STIPITIPELLIS hyphae thin-walled, 6.2–12.5 µm diam., filled with light brown content. VOLVA hyphae undifferentiated, septate, loosely interwoven, 2.5–10.0 µm diam., thin-walled, colorless to slightly yellowish. NO CLAMP CONNECTIONS seen in all parts examined.

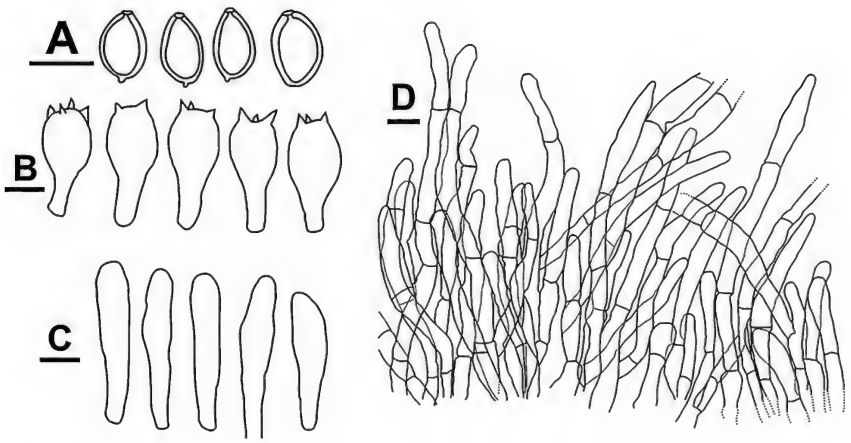

Fig. 3. *Macrolepiota dunensis* (holotype, SP466944). A. Basidiospores; B. Basidia; C. Cheilocystidia; D. Pileipellis elements. Scale bars = 10 µm. Line drawings: D.S. Freitas.

Ecology & Distribution—Solitary on sandy soil. In a fragment of shrubby-arboreal stratum on dunes, part of the Atlantic Forest domain in a 'restinga' (tropical coastal vegetation) area.

Comments—*Macrolepiota dunensis* is characterized by a dark brown pileus fully covered with appressed fibrils up to two-thirds from the center and with no velar remnants on the surface, a short stipe, cheilocystidia with slightly thickened walls towards the apex, and short individual terminal elements in the pileus covering. These and other additional morphological characters allied to the phylogenetic position of the sampled taxa (Fig. 1), distinguish *M. dunensis* from the other volvate *Macrolepiota* as discussed below.

Macrolepiota pulchella differs in its pileus with triangular brown-gray squamules at center, longer (≤130 mm) stipe, apical annulus, white membranous volva, slightly shorter (18–40 µm) cheilocystidia with verrucose apices, and pileus covering composed of some pyriform and wider (23–40 µm diam.) hyphae or sometimes walls encrusted with brown pigments (Heinemann & de Meijer 1996).

Macrolepiota eucharis differs in its pileus with black squamules on a brown-gray background, longer stipe (≤140 mm), slightly larger (10.8–15.5 × 7.1–9.1 µm) basidiospores, and pileus surface with shorter and wider (20–80 × 6.0–14 µm) terminal elements (Vellinga 2003).

Macrolepiota brunnescens differs in its broader (<120 mm) pileus partly covered by white woolly-membranous veil remnants, longer (115 mm) white stipe, longer (35–44 µm) basidia that have (sometimes) a brown resinous encrustation or (rarely) brown contents, and an apparent absence of cystidia (Singer 1959).

Macrolepiota velosa differs in its pileus surface with some purplish squamules and white to dirty white universal veil patches, much longer (100–170 mm) purplish brown tinged stipe, longer and narrower (44–68 × 4.5–7.5 µm) cheilocystidia with refractive apices and grayish-granular contents, and pileus covering with wider (25 µm diam.) terminal elements (Vellinga & Yang 2003).

Macrolepiota rhodosperma var. *velicopia* differs in its larger (≤150 mm diam.) pileus with white to dirty white membranous velar patches on the surface; much longer (≤180 mm) stipe; larger ((13–)13.5–15(–16.5) × 9–10.5(–11.2) µm) basidiospores; variously shaped cheilocystidia (narrowly clavate to sub-fusiform, lageniform, utriform, or cylindrical) that are sometimes apically capitulate, basally septate, or (rarely) with apical excrescences; and in the pileus covering much longer (≤250 µm) individual terminal elements with several secondary septa (pseudosepta) and some very thick-walled (1.5–2 µm) hyphae (Vizzini & al. 2011).

Finally, our other newly described taxon, *M. sabulosa* var. *velistellaris*, is distinguished by its slightly rimose pileus surface cracking into very small squamules towards the margin, slightly longer (95 mm) and distinctly squamulose stipe, uniformly thin-walled cheilocystidia, and stipe covering with distinct and ascendant terminal elements (see complete taxonomic description below).

Macrolepiota pulchella de Meijer & Vellinga, Mycotaxon 85:184, 2003. FIGS 2B,E, 4

≡ *Lepiotella brunnea* Rick, Lilloa 2: 251. 1938.

≡ *Volvolepiota brunnea* (Rick) Singer, Bol. Soc. Arg. Bot. 8: 12. 1959.

"*Chlorophyllum pulchellum*" Gimenes & Capelari, [unpublished master's thesis]. 2007.

PILEUS 32–68 mm diam., conic to convex, light brown ($A_{70}M_{70}C_{60}$; $N_{60}A_{40}M_{40}$) to slightly greyish brown ($N_{80}Y_{50}M_{40}$), dark brown ($A_{99}M_{90}C_{80}$; $N_{90}Y_{50}M_{40}$) on the entire and prominent umbo, margin entire or sometimes with inconspicuous velar remnants, surface completely scaly on fibrillose background and covered with dark brown ($A_{99}M_{70}C_{70}$; $N_{90}Y_{40}M_{40}$) scales (larger at center, smaller toward the edge). Context white, 3.5 mm thick. LAMELLAE free, remote, crowded, white to light cream ($N_{00}Y_{10}M_{00}$), with lamellulae. Stipe

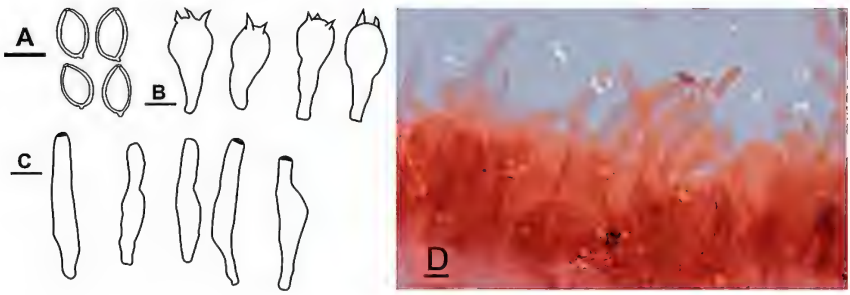

Fig. 4. *Macrolepiota pulchella* (SP466949). A. Basidiospores; B. Basidia; C. Cheilocystidia; D. Pileipellis elements. Scale bars = 10 µm. Line drawings: D.S. Freitas.

75–135 × 3–3.5(apex)/5–10(base) mm, central, dark brown at base and slightly lighter towards the apex, covered by inconspicuous zigzag bands mainly on central part, almost cylindrical or slightly tapering upwards with a subbulbous base, fibrous. Annulus apical, membranous, whitish upperside with brown edge, margin slightly splitting. Volva saccate, membranous with a cottony base, entire white. Odor and taste not recorded. Spore print white.

Basidiospores [187/9/9] (8.7–)9.0–13.7(–15.0) × (6.2–)7.5–10.0(–11.2) µm (Q = 1.1–2.0; Qm = 1.5; Lm = 12.2 µm; Wm = 8.4 µm), subglobose, broadly ellipsoid, ellipsoid, elongate, smooth, thick-walled, hyaline, with a slightly truncate germ pore covered by a hyaline cap, some with inconspicuous apiculus, dextrinoid in Melzer's and metachromatic in Cresyl blue. Basidia 22–40 × 7.5–15.0 µm clavate, hyaline, thin-walled or slightly thickened at apex, 2–3–4-spored. Cheilocystidia 27–55 × 6.2–10.0 µm, cylindrical to slightly clavate, hyaline, thin-walled, some apically encrusted. Hymenophoral trama irregular, hyphae thin-walled, 3.7–16.2 µm diam., hyaline. Pileus trama hyphae thin-walled, 3.7–18.7 µm diam., hyaline to slightly light brown. Pileus covering an intricate trichoderm, composed of thin-walled apically rounded cylindrical elements with light brown contents, terminal elements 72–157 × 5.0–7.5 µm; toward the center intermixed with clavate or pyriform elements. Stipitipellis hyphae thin-walled, 3.7–18.7 µm diam., with brown contents. Volva hyphae undifferentiated, 2.5–8.7 µm diam., thin-walled, septate, loosely interwoven, colorless. Clamp connections not seen in all parts examined.

Ecology & Distribution—Solitary on soil. In a 'seasonal semi-deciduous forest' fragment, part of the Atlantic Forest domain.

Specimens examined—**BRAZIL. São Paulo state: São Paulo:** Itapecerica da Serra, 10 July 1997, A.M. Gugliotta AMG945 (SP466950); 24 July 1997, M. Capelari

& A.M. Gugliotta MC3955 (SP466951); Parque Estadual das Fontes do Ipiranga, 9 October 2001, U.C. Peixoto PEFI13/2001 (SP381604); 21 September 2005, C. Puccinelli & U.C. Peixoto CP 161 (SP466948); 3 October 2006, C. Puccinelli CP193 (SP381606); 13 August 2006, L.J. Gimenes LJG130/06 (SP381605); 13 November 2007, Menolli Jr. & F. Karstedt NMJ155 (SP466946); 19 October 2011, J.J.S. Oliveira & P.O. Ventura JJSO386 (SP466949; GenBank MG136891); Parque Estadual da Cantareira, 23 October 2008, M. Capelari & L.A.S. Ramos MC4411 (SP466947; GenBank MG136894).

Comments—The morphological features observed in new and old collections confirm the occurrence of *M. pulchella* from São Paulo state, Southeast Brazil, as first noted by Bononi & al. (1981, as *V. brunnea*). However, the collection recorded by Bononi & al. (1981) does not represent *M. pulchella; Macrolepiota* sp. (SP61013) has much larger basidiospores (13.5–16.5 × 9.0–12.0 µm; Q= 1.3–1.6 µm; Qm = 1.4 µm; Lm = 14.9 µm; Wm = 10.6 µm), a longer (approx. 315 mm) stipe, and no evidence of a volva (or any velar remnants) on the stipe base.

In addition to the São Paulo state record, Gimenes (2007) recorded *M. pulchella* (as "*Chlorophyllum pulchellum*") from the same area—Parque Estadual das Fontes do Ipiranga—where we found most of our collections. Gimenes (2007) justified the transfer to *Chlorophyllum* based on an unresolved LSU sequence analysis and the morphology of the pileus covering (described as hymenidermal) and basidiospores (described as lacking the characteristic hyaline cap on the germ pore). However, our revision of the collections studied by Gimenes (2007) plus our examination of new collections and the molecular support confirms the identity of all materials as *Macrolepiota pulchella.*

The most complete morphological study of *M. pulchella* was published by Heinemann & de Meijer (1996) based on a unique basidioma (de Meijer 2935) from Paraná state in South Brazil. They also cited three other collections, drawing attention to minor morphological variations such as the pileus color, context, and squamules; stipe base shape; stability of the annulus; and a volva that can be inconspicuous.

Compared to our *M. pulchella* collections, Heinemann & de Meijer (1996) reported smaller basidiospores ((8.6–)9.41–10.3 × 5.8–6.19(–6.8) µm) and shorter basidia (24–26 µm) and cheilocystidia (18–40 µm), with the cheilocystidia (4–8(–10) µm diam.) sometimes narrowly lanceolate or slightly verrucose at apex. They also described the pileus covering as:

"... in the center, palisadic, formed of erect hyphae, 5–10 µm diam., with some much larger, e.g. 23–40 µm broad, pyriform elements which are seen at various

distance from surface; this structure is covered with a layer, 10–35 µm thick, made up of cylindrical, ± radially oriented hyphae, 3–7 µm diam., originating probably from the underlying structure and constituting a general veil; in the periphery, squamules made of cylindrical parallel hyphae, 6–11 µm diam., wall with brown incrusted pigment."

We wish to highlight that among our specimens we observed the characteristic trichodermal pileus covering with long, non-encrusted terminal elements (72–157 × 5.0–7.5 µm). We also noted i) only 2–spored basidia in NMJ155, (23.7–27.5 × 10.0–12.5 µm) similar in size to those described by Heinemann & de Meijer (1996), and basidiospores ((9.5–)10.0–11.5(–12.0) × 7.5–9.0 µm) slightly smaller than the other collections; ii) in JJSO386, the presence of clavate to pyriform elements in the pileus disc covering; and iii) in JJSO386, apically encrusted cheilocystidia possibly corresponding to what Heinemann & de Meijer (1996) described as "slightly verrucose," which, however, does not seem to be a constant character for the species.

Considering that Heinemann & de Meijer (1996) based their description on a single collection, we feel we have accurately described additional morphological characters for *M. pulchella* while providing the first molecular data to contribute to a phylogeny of volvate *Macrolepiota* from Brazil. Additional morphological examinations are needed to confirm the identity of the specimens named by Fazolino Perez & al. (2018) as *Macrolepiota* sp. 3, but our molecular analyses suggest they represent *M. pulchella,* which would be a first record of *M. pulchella* from Rio Grande do Norte state in Northeast Brazil.

Macrolepiota sabulosa var. ***velistellaris*** D.S. Freitas & Menolli, **var. nov.**

MB 823089 FIGS 2C,F, 5

Differs from the non-volvate autonymous variety *Macrolepiota sabulosa* var. *sabulosa* by the presence of a distinct volva on the stipe base.

TYPE—Brazil. Rio Grande do Norte: Baía Formosa, Mata Estrela, Reserva Particular de Patrimônio Natural Mata Estrela, 14 July 2010, Menolli Jr. & al. NMJ185 (**Holotype**, SP466945; GenBank MG136893).

ETYMOLOGY—*veli* (L. for veil), referring to the presence of a volva and velar remnants on the stipe base; *stellaris* (L. for star-shaped), referring to both the star-like arrangement of the pileus squamules and Mata Estrela (Star Forest), the private area where the holotype was collected.

PILEUS 62 mm, plano-convex, with an inconspicuous umbo, dark brown ($N_{80}A_{60}M_{50}$) at center, covering slightly rimose and cracking into very small squamules on one-third distant from the umbo before dissociating radially

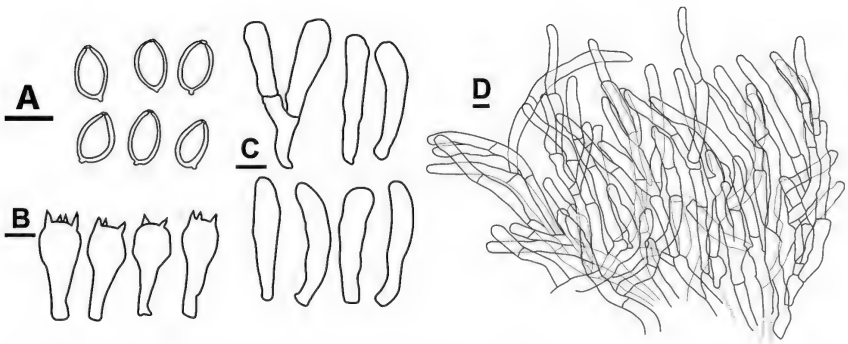

Fig. 5. *Macrolepiota sabulosa* var. *velistellaris* (holotype, SP466945). a. Basidiospores; b. Basidia; c. Cheilocystidia; d. Pileipellis elements. Scale bars = 10 μm. Line drawings: D.S. Freitas.

towards the margin into fibrils on cream background. Lamellae free, crowded, white, with lamellulae. Stipe 95 × 6(apex)/7(base) mm, central, almost cylindrical, covered throughout with small scales forming distinct zigzag bands that concolourous with the pileus disc, lighter at the apex. Annulus apical, membranous, movable, whitish upperside with brown edge. Volva limbate, membranous with a cottony base, dirty white. Context, odor, taste and spore print color not recorded.

Basidiospores [25/1/1] 9.5–12.0 × 6.0–8.0 μm (Q = 1.3–1.8; Qm = 1.5; Lm = 10.7 μm; Wm = 6.9 μm), ellipsoid to elongated, rarely broadly ellipsoid, smooth, thick-walled, hyaline, with distinctive apiculus and an apical central truncate germ pore covered by a hyaline cap, dextrinoid in Melzer's and metachromatic in Cresyl blue. Basidia 29–36 × 11.0–14.0 μm, clavate, hyaline, thin-walled, 2–3–4-spored. Cheilocystidia 25–45 × 7.5–10.0 μm, cylindrical to narrowly clavate, hyaline, thin-walled, sometimes in clusters. Hymenophoral trama irregular, hyphae thin-walled, 5.0–12.5 μm diam., hyaline. Pileus trama hyphae thin-walled, 6.2–11.2 μm diam., hyaline to brownish. Pileus covering an intricate trichoderm, composed of thin-walled cylindrical elements with light brown content, terminal elements measuring 118–193 × 5.0–8.7 μm. Stipitipellis hyphae thin-walled, 5.0–11.2 μm diam., filled with light brown content; terminal elements elongated, conspicuous, ascendant, 6.2–11.2 μm diam. Volva hyphae undifferentiated, septate, moderately branched, loosely interwoven, thin-walled, 3.7–10.0 μm diam., colorless to slightly yellowish. Clamp connections not seen in all parts examined.

Ecology & Distribution—Solitary on soil. In a 'dense ombrophilous forest' fragment within the Atlantic Forest domain.

Comments—*Macrolepiota sabulosa* var. *velistellaris* is proposed as new based on the presence of a distinct volva on the stipe base. Fazolino Perez & al. (2018) described *M. sabulosa* based on two collections from the same state in Northeast Brazil where we collected the new variety. They distinguished *M. sabulosa* from *M. pulchella* due to the presence of a volva on *M. pulchella,* a character neither shown in their illustrations nor mentioned in the protologue of *M. sabulosa*. Additionally, *M. sabulosa* var. *sabulosa* has more robust basidiomata (pileus 130–140 mm, stipe 165–175 × 11–12 mm), shorter terminal elements in the pileus covering (28–63.5 × 5.5–8.5 µm), and clamp connections on hyphal septa in the stipe context and at the bases of basidia, basidioles, and cheilocystidia (Fazolino Perez & al. 2018).

Macrolepiota sabulosa var. *velistellaris* morphologically differs from *M. dunensis* as previously discussed in the notes under *M. dunensis*, as well as from *M. pulchella*, *M. eucharis*, *M. brunnescens*, *M. velosa,* and *M. rhodosperma* var. *velicopia*.

Macrolepiota pulchella has differently arranged pileus squamules, cheilocystidia with a slightly verrucose apex, and a stipe surface composed of brown hyphae (3–9 µm diam.) in a pseudoparenchyma under a palisade structure (no mention of distinct or ascendant terminal elements; Heinemann & de Meijer 1996).

Macrolepiota eucharis is distinguished by its brown-gray pileus background, longer stipe (≤140 mm), slightly larger (10.8–15.5 × 7.1–9.1 µm) basidiospores, mostly 4-spored basidia, slightly fusiform and slightly thick-walled cheilocystidia, and wider (≤14 µm diam.) terminal pileipellis elements (Vellinga 2003).

Macrolepiota brunnescens has a broader (≤120 mm) pileus covered by a woolly whitish membrane, undecorated and initially white stipe, encrusted and pigmented basidia, and (apparently) no cystidia (Singer 1959).

Macrolepiota velosa differs in its purplish tinged pileus squamules, much longer (100–170 mm) stipe, longer and narrower (44–68 × 4.5–7.5 µm) cheilocystidia with a distinct apex, and shorter (≤100 µm) pileipellis terminal elements (Vellinga & Yang 2003).

Macrolepiota rhodosperma var. *velicopia* has a much broader (≤150 mm diam.) pileus covered with white universal veil remnants, much longer (≤180 mm) stipe with a fringed annulus, larger ((13–)13.5–15(–16.5) × 9–10.5(–11.2) µm)

basidiospores, differently shaped cheilocystidia (varying among narrowly clavate to sub-fusiform, lageniform, utriform or cylindrical), longer (≤250 µm) pileipellis elements that are normally thick-walled (1.5–2 µm thick) and provided with internal pseudosepta (Vizzini & al. 2011).

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Literature cited

Bas C. 1969. Morphology and subdivision of *Amanita* and a monograph of its section *Lepidella*. Persoonia 5: 285–579.

Bononi VLR, Trufem SFB, Grandi RAP. 1981. Fungos macroscópicos do Parque Estadual das Fontes do Ipiranga, São Paulo, Brasil, depositados no Herbário do Instituto de Botânica de São Paulo. Rickia 9: 37–53.

de Meijer AAR. 2006. Preliminary list of macromycetes from the Brazilian State of Paraná. Boletim do Museu Botânico Municipal 68: 1–55.

Fazolino Perez E, Suaza Blandón SC, Alves-Silva G, Lechner BE, Silveira RMB. 2018. Taxonomy and phylogeny of *Macrolepiota*: two new species from Brazil. Mycologia 110: 930–940. https://doi.org/10.1080/00275514.2018.1500848

Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and rusts. Molecular Ecology 2: 113–118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x

Ge ZW, Yang ZL, Vellinga EC. 2010. The genus *Macrolepiota* (*Agaricaceae, Basidiomycota*) in China. Fungal Diversity 45: 81–98. https://doi.org/10.1007/s13225-010-0062-0

Gimenes LJ. 2007. A tribo *Leucocoprineae* (*Agaricaceae*) no Parque Estadual das Fontes do Ipiranga, São Paulo, SP, Brasil. [Unpublished Master's Dissertation], Instituto de Botânica de São Paulo. 84 p.

Heinemann P, de Meijer AAR. 1996. The status of *Volvolepiota* Sing. Bulletin du Jardin Botanique National de Belgique 65: 405–412. https://doi.org/10.2307/3668462

Hurvich CM, Tsai C-L. 1991. Bias of the corrected AIC criterion for underfitted regression and time series models. Biometrika 78: 499–509. https://doi.org/10.1093/biomet/78.3.499

Justo A, Vizzini A, Minnis AM, Menolli Jr N, Capelari M, Rodríguez O, Malysheva E, Contu M, Ghignone S, Hibbett DS. 2011a. Phylogeny of the *Pluteaceae* (*Agaricales, Basidiomycota*): taxonomy and character evolution. Fungal Biology 115: 1–20. https://doi.org/10.1016/j.funbio.2010.09.012

Justo A, Minnis AM, Ghignone S, Menolli Jr N, Capelari M, Rodríguez O, Malysheva E, Contu M, Vizzini A. 2011b. Species recognition in *Pluteus* and *Volvopluteus* (*Pluteaceae*, *Agaricales*): morphology, geography and phylogeny. Mycological Progress 10: 453–479. https://doi.org/10.1007/s11557-010-0716-z

Katoh K, Toh H. 2008. Recent developments in the MAFFT multiple sequence alignment program. Briefings in Bioinformatics 9: 286–298. https://doi.org/10.1093/bib/bbn013

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A. 2012. Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28: 1647–1649. https://doi.org/10.1093/bioinformatics/bts199

Küppers H. 1979. Atlas de los colores: más de 5500 matices con su caracterización y las instrucciones para su mezcla. Editorial Blume, Barcelona.

Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B. 2017. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Molecular Biology and Evolution 34: 772–773. https://doi.org/10.1093/molbev/msw260

Lebel T, Syme A. 2012. Sequestrate species of *Agaricus* and *Macrolepiota* from Australia: new species and combinations and their position in a calibrated phylogeny. Mycologia 104: 496–520. https://doi.org/10.3852/11-092

Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES science gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments Workshop (GCE). New Orleans, Louisiana. 8 p. https://doi.org/10.1109/GCE.2010.5676129

Rick J. 1938. Agarici Riograndenses II. Lilloa 2: 251–316.

Rick J. 1961. Basidiomycetes Eubasidii no Rio Grande do Sul, Brasília. Iheringia 8: 296–450.

Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542. https://doi.org/10.1093/sysbio/sys029

Singer R. 1948. New and interesting species of *Basidiomycetes*. II. Papers of the Michigan Academy of Sciences 32: 103–150.

Singer R. 1954. ["1953"] Type studies on *Basidiomycetes* VI. Lilloa 26: 57–159.

Singer R. 1959. Dos generos de hongos nuevos para Argentina. Boletín de la Sociedad Argentina de Botánica 8: 9–13.

Singer R. 1986. The *Agaricales* in modern taxonomy. 4th edition. Koenigstein: Koeltz Scientific Books.

Stamatakis A. 2014. RAxML Version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30: 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Strongman DB, Wang J, Xu S. 2010. New trichomycetes from western China. Mycologia 101: 174–184.

Thiers B. 2018 [continuously updated]. Index Herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. Accessed Aug 2018: http://sweetgum.nybg.org/ih/

Vellinga EC. 1988. Glossary. 54–64, in: C Bas & al. (eds). Flora Agaricina Neerlandica, vol. 1. A.A. Balkema Publishers, Amsterdam/Brookfield.

Vellinga EC. 2003. *Chlorophyllum* and *Macrolepiota* (*Agaricaceae*) in Australia. Australian Systematic Botany 16: 361–370. https://doi.org/10.1071/sb02013

Vellinga EC, Noordeloos ME. 2001. Glossary. 6–11, in: ME Noordeloos & al. (eds). Flora Agaricina Neerlandica, vol. 5. A.A. Balkema Publishers, Lisse/Abingdon/Exton/Tokyo.

Vellinga EC, Yang ZL. 2003. *Volvolepiota* and *Macrolepiota—Macrolepiota velosa*, a new species from China. Mycotaxon 85: 183–186.

Vellinga EC, Kok RPJ, Bruns TD. 2003. Phylogeny and taxonomy of *Macrolepiota* (*Agaricaceae*). Mycologia 95: 442–456. https://doi.org/10.1080/15572536.2004.11833089

Veloso HP, Rangel-Filho ALR, Lima JCA. 1991. Classificação da vegetação brasileira adaptada a um sistema universal. Rio de Janeiro: IBGE, Departamento de Recursos Naturais e Estudos Ambientais.

Vizzini A, Contu M, Ghignone S, Vellinga E. 2011. A new volvate *Macrolepiota* (*Agaricomycetes*, *Agaricales*) from Italy, with observations on the *M. procera* complex. Mycotaxon 117: 149–164. http://dx.doi.org/10.5248/117.149

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. 315–322, in: MA Innis & al. (eds). PCR Protocols: a guide to methods and applications. San Diego, Academic Press. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

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Inocybe caroticolor from oak forests of Pakistan

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Abstract—Basidiomata of *Inocybe caroticolor* were collected from two different *Quercus* forests in Swat, KP, Pakistan. Based on morphology and molecular phylogenetic analyses of ITS and 28S rDNA regions, these basidiomata were found to represent *I. caroticolor* and placed within the *Inocybe viscata* group. Our *I. caroticolor* specimens are molecularly supported as conspecific with specimens from China and represent a first species record from Pakistan.

Key words—GenBank, Himalayan, *Inocybaceae*, LSU

Introduction

The genus *Inocybe* (Fr.) Fr. (*Inocybaceae*) is distributed worldwide and covers a wide geographical range from tropical to arctic-alpine regions (Favre 1955; Horak 1979, 1980, 1981, 1987; Kühner 1988; Buyck & Eyssartier 1999; Watling 2001; Matheny & al. 2003; Jacobsson 2008). As one of the larger ectomycorrhizal genera in *Agaricales, Inocybe* is an important component of ectomycorrhizal temperate and boreal forest communities in alpine and arctic habitats (Cripps & al. 2010, Kokkonen & Vauras 2012).

Between 70% and 80% of the species in *Inocybaceae* have been described in associations primarily with ectomycorrhizal plant families *Fagaceae*, *Pinaceae*, and *Salicaceae* (Kirk & al. 2008). *Quercus* (oak), an important genus in the *Fagaceae,* is also known as the 'king of trees' due to its vital ecological role. In Pakistan, oaks are among the dominant vascular plants of the Himalayas, ranging from the subtropical to the sub-alpine zones

(Shrestha 2003). Himalayan oaks are evergreen, mostly gregarious, medium- to large-sized trees distributed at elevations of 800–3800 m a.s.l. throughout the Himalayan region. According to Alamgir (2004), established oak forests cover approximately 16,800 ha, making *Quercus* an important genus in temperate regions in Pakistan. Swat lies in Himalaya and its mycoflora is poorly known.

More than 850 *Inocybe* species have been reported worldwide (Matheny & al. 2009, 2012; Kobayashi & Onishi 2010; Horak & al. 2015; Jabeen & al. 2016). This number is increasing; during 2015–18 newly described species of *Inocybaceae* included 100 from Australia (Matheny & Bougher 2017), 19 from New Zealand (Horak 2018), 17 from India (Latha & Manimohan 2017), and 13 from Thailand and Malaysia (Horak & al. 2015). Species are also regularly being described in northern and southern Europe (e.g., Kokkonen & Vauras 2012; Esteve-Raventós & al. 2015, 2016, 2017; Bizio & al. 2016; Franchi & al. 2016; Vauras & Larsson 2016). *Inocybe* has been widely explored throughout Asia in China (Fan & Bau 2013, 2014), India (Vrinda & al. 1996, 1997; Pradeep & Vrinda 2007, 2010; Latha & Manimohan 2015, 2016, 2017; Tibpromma & al. 2017), Japan (Kobayashi & Courtecuisse 1993, 2000; Kobayashi & Hongo 1993; Kobayashi 2009; Kobayashi & Onishi 2010), and the Himalayas (Horak 1981). Only a few studies of the genus, however, have been undertaken in Pakistan. Since Ahmad & al. (1997) published their first report of *Inocybe* from Pakistan, 29 *Inocybe* species have been reported from different parts of the country (Ilyas & al. 2013, Saba & al. 2015, Jabeen & al. 2016, Farooqi & al. 2017, Naseer & al. 2018, Ullah & al. 2018), of which four—*I. ahmadii* Farooqi & al., *I. kohistanensis* Jabeen & al., *I. pakistanensis* Z. Ullah & al., and *I. shawarensis* Naseer & Khalid—were described as new to science (Jabeen & al. 2016, Farooqi & al. 2017, Naseer & al. 2018, Ullah & al. 2018).

During 2014–16 we collected specimens on field research trips while studying fungal communities associated with the oak forests of Pakistan. At that time we encountered in Swat an *Inocybe* species previously unknown from Pakistan. Its bright red carrot color easily distinguished this fungus in the field, and in the laboratory, we identified it as *Inocybe caroticolor*. Here we present a taxonomic description of our new Pakistani collections with the results of our ITS1-5.8S-ITS2 and 28S rDNA sequence analyses phylogenetically placing this species in the *Inocybe viscata* group of *Inocybe* clade (Matheny & Bougher 2017).

Material & methods

Collection & morphological examination

Specimens were collected from two different locations from the moist temperate forest of Toa, Swat as described in Naseer & al. (2017) and Shawar Valley as described in Naseer & al. (2018). These forests are dominated by *Quercus incana* Roxb. nom. illeg. [= *Q. oblongata* D. Don] and to a lesser extent *Q. dilatata* Royle. Gross morphological characters were recorded in the field and photographed using a Nikon D70S digital camera. Basidiomata were wrapped in aluminium foil and kept separately in a collection box to avoid mixing or crushing. Specimens were dried, sealed in plastic bag, and deposited in Lahore Herbarium, Department of Botany, University of the Punjab, Lahore, Pakistan (LAH). Colors were designated by mColorMeter. For anatomical examination, tissues from lamellae, pileipellis and stipitipellis were mounted on glass slides and observed in Phloxine (1%), Melzer's reagent, and KOH (5%) for pigmented hyphae and walls of cystidia using a Meiji Techno MX4300H microscope. Dimensions were measured for basidiospores, basidia and other elements from basidiomata under the light microscope equipped with a camera lucida.

Molecular phylogenetic analyses

DNA was extracted from lamellae of basidiomata following a modified CTAB method (Bruns 1995). The ITS region was amplified using primer pairs ITS1F/ITS4 (White & al. 1990) and the nrDNA 28S region using primer pairs LR0R/LR5 (Vilgalys & Hester 1990). Amplification parameters proceeded with an initial denaturation step at 94°C for 4 min, followed by 34 cycles of 94°C for 40 sec, 55°C (ITS) or 52°C (28S) for 40 sec, and 72°C for 1 min, and a final elongation step at 72°C for 8 min. The PCR products were purified, and Macrogen (South Korea) conducted bidirectional sequencing. The consensus sequences were subjected to BLAST, and compared with available GenBank sequences. The newly generated sequences were aligned with the closest matching NCBI sequences using MUSCLE v3.6 (Edgar 2004) and manually adjusted where necessary. ML tree generation and bootstrap analyses were performed for combined ITS and 28S data set on CIPRES Gateway (Miller & al. 2010). A maximum likelihood (ML) tree was inferred using RAxML-HPC2 v 8.1.11 (Stamatakis 2014) with a GTR + gamma model of nucleotide substitution. One thousand bootstrap iterations were performed with rapid bootstrapping. Significant support was considered to be ≥70%. Sequences generated in this study were submitted to GenBank.

Taxonomy

Inocybe caroticolor T. Bau & Y.G. Fan, Mycotaxon 123: 170 (2013) Figs 1, 2

Basidiomata small. Pileus 12–32 mm diam., bright red (2.4YR 3.1/9.3) in centre becoming yellowish (0.5Y 6.3/7) with patches of reddish orange; conical when young, then plano-convex, with an obtuse umbo, slightly rimulose, surface dry with appressed squamules over disc, radially fibrillose,

Fig. 1. *Inocybe caroticolor*. Basidiomata.
A, B. LAH35266; C, D. LAH35268; E–G. LAH35269.
Scale bars: A, B, E = 0.7 cm; C, D, F = 0.33 cm; G = 0.5 cm

rimose, margins inrolled (young) to incurved (mature). Lamellae light yellow (4.6Y 8.9/4.2) when young to yellowish orange (2.6YR 2.5/5.4) when mature, numbering 45–60, regularly arranged, adnexed, slightly close to crowded, 2–3 mm deep, edges even, smooth; lamellulae ¼ length of lamellae, single-tiered and alternating with lamellae. Stipe 32 × 4 mm, central, cylindrical, with subbulbous to non-marginate bulbous base, fibrillose, pruinose entire length, densely so at apex, longitudinally striate; yellow (4Y 6.2/6.5) in the middle region, and reddish orange (2.4YR 3.1/9.3) at apex, basal tomentum white.

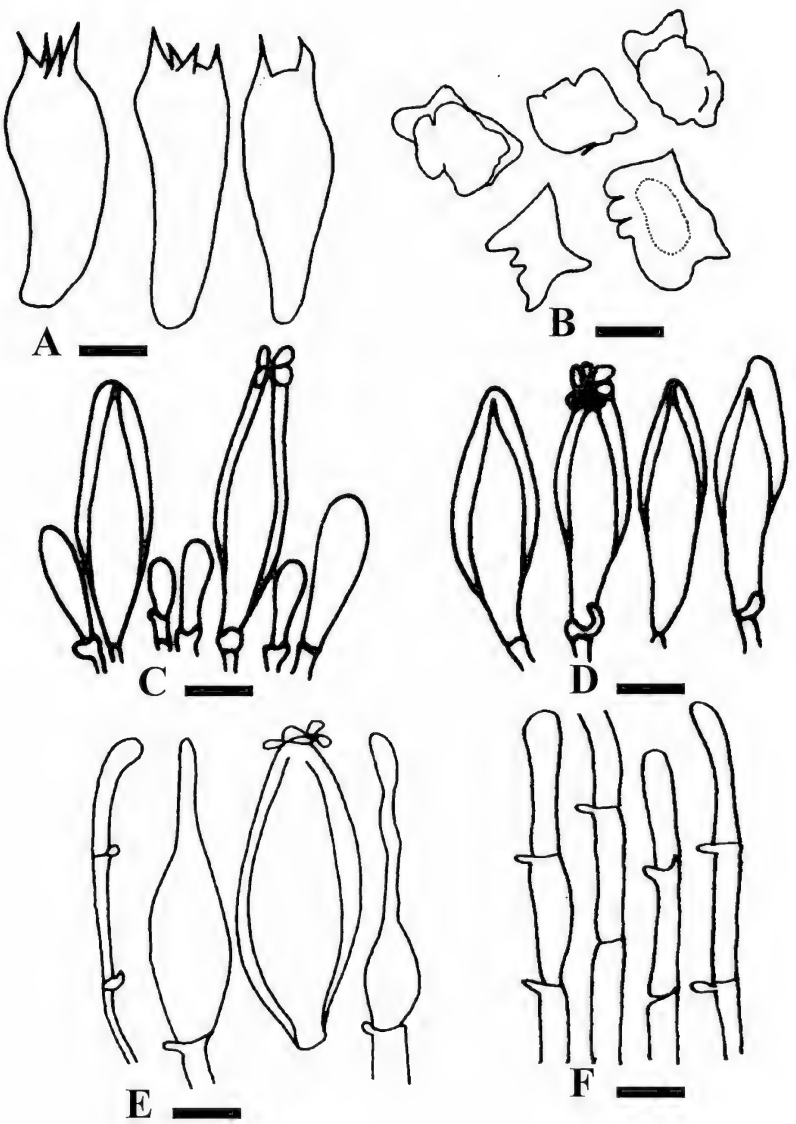

Fig. 2. *Inocybe caroticolor* (LAH35266). A. Basidia; B. Basidiospores; C. Cheilocystidia; D. Pleurocystidia; E. Caulocystidia with paracystidia; F. Pileipellis. Scale bars: A = 7.90 µm; B = 4.39 µm; C = 9.54 µm; D, E = 14 µm; F = 6.7 µm.

Basidiospores [n/b/p = 60/3/3], (6.3–)6.5–9.3(–9.5) × (4.5–)4.7–6.0 (–6.1) µm, mean = 7.5 × 5.2 µm, Q = (1.43–)1.47–1.58(–1.61), pale brown in 5% KOH, weakly to strongly nodulose. Basidia 25–36 × 5–8 µm, clavate, 4-spored, with yellowish pigments. Pleurocystidia 47–63 × 12–15 µm, fusiform, with crystalliferous apex, yellow in 5% KOH, thick walled, obtuse base. Cheilocystidia 48–62 × 12–15 µm, fusiform, some cylindrical. Caulocystidia 49–75 × 9–13 µm, variably shaped, abundant, thin-walled. Pileipellis 0.2–6.5 µm, a cutis of cylindrical hyphae, smooth, pigmented, thin-walled. Stipitipellis 2–4.5 µm, a cutis composed of regularly arranged hyphae, filamentous, smooth, thin- to moderately thick-walled. Clamp connections present.

Material examined: , Toa, 2100 m a.s.l, on soil under *Quercus oblongata*, 15 July 2015, Arooj Naseer, Abdul Rehman Niazi & Abdul Nasir Khalid AST47 (LAH35266; GenBank MH473148, MH536985); , Shawar Valley, 2100 m a.s.l, solitary on soil under *Q. dilatata* and *Q. oblongata* 14 July 2014, Arooj Naseer & Abdul Nasir Khalid ASSW11 (LAH35268; GenBank MH473144, MH536984); 15 Aug 2015, Arooj Naseer & Abdul Nasir Khalid ASS6 (LAH35267; GenBank MH473145, MH536986); 25 August 2014, Arooj Naseer & Abdul Nasir Khalid ASSW29 (LAH35269; GenBank MH473146, MH536983).

Molecular phylogenetic characterization

Sequencing of the ITS PCR products from *Inocybe caroticolor* specimens yielded 580–745 base pairs. NCBI BLAST searches of consensus sequences from four collections showed these sequences as 99% identical with *Inocybe caroticolor* (JX025772, JX025773 and JX025774) from China (100% query coverage; 0.0 E value). These sequences also showed a 93% identity with *Inocybe* sp. TR198-03 (KP636865) from Papua New Guinea.

Sequencing of the 28S PCR products yielded 867–980 base pairs. Consensus sequences of the 835 base pairs BLAST searched at NCBI showed 97% identity with *Inocybe* sp. REH7418 (JN974931) from an oak forest in Costa Rica and *Inocybe* sp.TR132-05 (KP171061) and *Inocybe* sp. TR88-06 (JN974992) from *Castanopsis* forests in Papua New Guinea with 100% query cover and 0.0 E value.

Sequences representing the *Inocybe viscata* group (*Inocybe* clade; Matheny & Bougher 2017), Indian species in a related clade (Latha & Manimohan 2017), and *Auritella dolichocystis* as outgroup were selected for preparation of the ITS/28S-based phylogram. Our newly generated sequences clustered with sequences from China with strong bootstrap value (Fig 3).

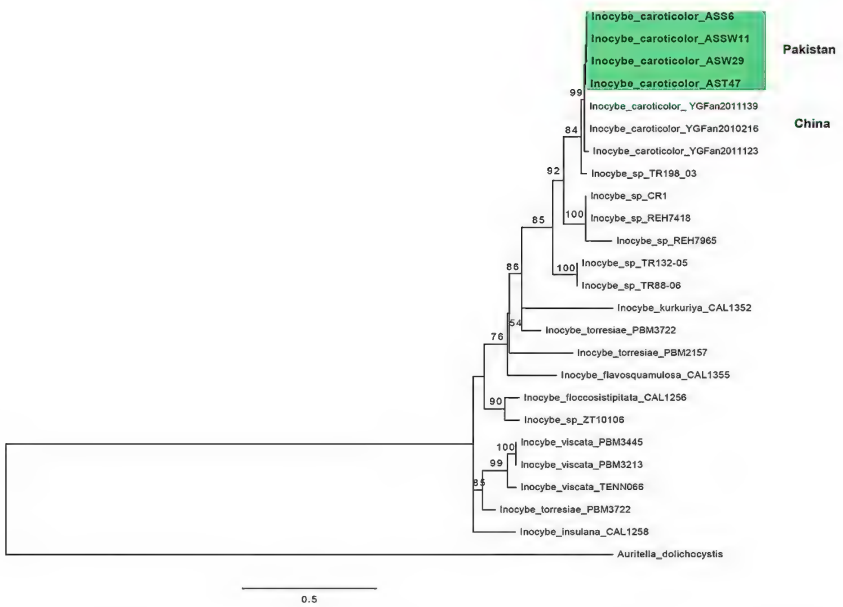

FIG. 3. ML phylogram of *Inocybe caroticolor* in the *I. viscata* group based on combined ITS and 28S nrDNA as generated with RAxML with 1000 bootstrap iterations. The letters in bold refer to sequences generated in this study, and sequences from Pakistan are highlighted in green.

Discussion

Inocybe caroticolor is characterized by the bright reddish carrot color of the basidiomata, nodulose basidiospores, and thick-walled hymenial cystidia. It can be easily recognized in the field by its bright red color, distinct aromatic odor, and entirely pruinose stipe. ITS sequence analysis clustered sequences from our Pakistani collections phylogenetically with those reported from China by Fan & Bau (2013), while the 28S phylogeny supported separation of the sequences from Pakistan in a separate clade from the sequences from *Inocybe* sp. TR88.06, *Inocybe* sp. TR132.05, and *Inocybe* sp. TR198.03 as observed in the ITS-based phylogram. However, as no other 28S-based sequences were present in GenBank, they were not included in a 28S-based phylogram.

The Pakistani collection of *I. caroticolor* differs somewhat morphologically from Chinese collection, which Fan & Bau (2013) described with a smaller

pileus and brighter red carrot color. In China, *I. caroticolor* has been found in association with *Quercus variabilis* Blume except for one collection found under *Pinus yunnanensis* Franch. (Fan & Bau 2013). During this study, all four collections from Pakistan were associated with *Q. oblongata* and *Q. dilatata* forests, indicating a strong preference for *Quercus*. This hypothesis is strengthened by the presence of other oak-specific species in the same clade (Matheny & Bougher 2017).

Our collections represent first record of *Inocybe caroticolor* for Pakistan. This species also exhibits a strong attraction for different oak species. In Pakistan, oak forests are being cut extensively for burning fuel and thus subject to decline. The presence of this unusual species may help develop an awareness of the need to conserve, establish, and reclaim these oak forests.

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Literature cited

Ahmad S, Iqbal SH, Khalid AN. 1997. Fungi of Pakistan. Lahore, Pakistan: Sultan Ahmad Mycological Society.

Alamgir KG. 2004. A study on the conditions, use, management and trends of major forest types in Chitral District. Report on Chitral Conservation Strategy and IUCN Sarhad Program, 111 p.

Bizio E, Ferisin G, Dovana F. 2016. *Inocybe costinitii*, a new species from the Istrian coast. Micologia e Vegetazione Mediterranea 31(2): 95–102.

Bruns TD. 1995. Thoughts on the processes that maintain local species diversity of ectomycorrhizal fungi. Plant and Soil 170: 63–73. https://doi.org/10.1007/BF02183055

Buyck B, Eyssartier G. 1999. Two new species of *Inocybe* (*Cortinariaceae*) from African woodland. Kew Bulletin 54: 675–681. https://doi.org/10.2307/4110863

Cripps CL, Larsson E, Horak E. 2010. Subgenus *Mallocybe* (*Inocybe*) in the Rocky Mountain alpine zone. North American Fungi 5(5): 97–126. https://doi.org/10.2509/naf2010.005.0057

Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32: 1792–1797. https://doi.org/10.1093/nar/gkh340

Esteve-Raventós F, Moreno G, Bizio E, Alvarado P. 2015. *Inocybe flavobrunnescens*, a new species in section *Marginatae*. Mycological Progress 14:14 [12 p.]. https://doi.org/10.1007/s11557-015-1036-0

Esteve-Raventós F, Moreno G, Alvarado P, Olariaga I. 2016. Unraveling the *Inocybe praetervisa* group through type studies and ITS data: *Inocybe praetervisoides* sp. nov. from the Mediterranean region. Mycologia, 108(1): 123–134. https://doi.org/10.3852/15-053

Esteve-Raventós F, Rodríguez-Campo FJ, Alvarado P. 2017. Fungal Planet 603 – 20 June 2017. *Inocybe parvicystis* F.J. Rodr.-Campo & Esteve-Rav., sp. nov. Persoonia 38: 338–339. https://doi.org/10.3767/003158517X698941

Fan YG, Bau T. 2013. Two striking *Inocybe* species from Yunnan Province, China. Mycotaxon 123: 169–181.https://doi.org/10.5248/123.169

Fan YG, Bau T. 2014. *Inocybe miyiensis*, a new two-spored species in section *Marginatae* from China. Nova Hedwigia 98: 179–185. https://doi.org/10.1127/0029-5035/2013/0135

Farooqi A, Aqdus F, Niazi AR, Jabeen S, Khalid AN. 2017. *Inocybe ahmadii* sp. nov. and a new record of *I. leptocystis* from Pakistan. Mycotaxon 132: 257–269. https://doi.org/10.5248/132.257

Favre J. 1955. Les champignons supérieurs de la zone alpine du Parc National Suisse. Ergebnisse der Wissenschaftlichen Untersuchung des Schweizerischen Nationalparkes 5:1–212.

Franchi P, Marchetti M, Papetti C. 2016. *Inocybe tiliae*, una nuova specie della Sezione *Marginatae*. Rivista di Micologia 59: 99–121.

Horak E. 1979. *Astrosporina* (*Agaricales*) in Indomalaya and Australasia. Persoonia 10: 157–205.

Horak E. 1980. *Inocybe* (*Agaricales*) in Indomalaya and Australasia. *Persoonia* 11: 1–37.

Horak E. 1981. On Himalayan species of *Astrosporina* and *Inocybe* (*Agaricales*). Persoonia 11: 303–310.

Horak E. 1987. *Astrosporina* in the alpine zone of the Swiss National Park (SNP) and adjacent regions. 205-234, in Arctic and Alpine Mycology II, Eds., G.A. Laursen, J.F. Ammirati, S.A. Redhead, Plenum Press, New York. 364p. https://doi.org/10.1007/978-1-4757-1939-0_14

Horak E. 2018. *Agaricales* (*Basidiomycota*) of New Zealand. 2. Brown spored genera p.p. Fungi of New Zealand/Ngā Hekaheka o Aotearoa, vol. 6. Westerdijk Biodiversity Series 16. 205 p.

Horak E, Matheny PB, Desjardin DE, Soytong K. 2015. The genus *Inocybe* (*Inocybaceae*, *Agaricales*, *Basidiomycota*) in Thailand and Malaysia. Phytotaxa 230: 201–238. https://doi.org/10.11646/phytotaxa.230.3.1

Ilyas S, Razaq A, Khalid AN. 2013. *Inocybe nitidiuscula* and its ectomycorrhizae with *Alnus nitida* from Galyat, Pakistan. Mycotaxon 124: 247–254. https://doi.org/10.5248/124.247

Jabeen S, Ahmad I, Rashed A, Khalid AN. 2016. *Inocybe kohistanensis*, a new species from Swat, Pakistan. Turkish Journal of Botany 40: 312–318. https://doi.org/10.3906/bot-1501-17

Jacobsson S. 2008. Key to *Inocybe*. 868–906, in: H Knudsen, J Vesterholt (eds.). Funga Nordica. Agaricoid, boletoid and cyphelloid genera. Nordsvamp, Copenhagen.

Kirk PM, Cannon PF, Minter DW, Stalpers JA. 2008. Dictionary of the Fungi (10th edn). CABI, Wallingford. 771 p.

Kobayashi T. 2009. Notes on the genus *Inocybe* of Japan: IV. Species having metuloids collected from Hokkaido, Honshu, and Kyushu. Mycoscience 50: 203–211. https://doi.org/10.1007/s10267-008-0472-y

Kobayashi T, Courtecuisse R. 1993. Two new species of *Inocybe* from Japan. Mycotaxon 46: 27–33.

Kobayashi T, Courtecuisse R. 2000. Two new species of *Inocybe*, section *Marginatae* (*Agaricales*, *Cortinariaceae*) from Japan. Mycoscience 41: 161–166. https://doi.org/10.1007/BF02464326

Kobayashi T, Hongo T. 1993. *Inocybe flocculosa* rediscovered in Japan. Transactions of the Mycological Society of Japan 34: 507–510.

Kobayashi T, Onishi S. 2010. *Inocybe sericella*, a new species of *Inocybe* sect. *Inocybe* [= *Cortinatae*] from Kobe, Japan. Nova Hedwigia 90: 227–232. https://doi.org/10.1127/0029-5035/2010/0090-0227

Kokkonen K, Vauras J. 2012. Eleven new boreal species of *Inocybe* with nodulose spores. Mycological Progress 11: 299–341. https://doi.org/10.1007/s11557-011-0783-9

Kühner R. 1988. Diagnoses de quelques nouveaux *Inocybes* récoltés en zone alpine de la Vanoise (Alpes françaises). Documents Mycologiques 19(74): 1–27.

Latha KPD, Manimohan P. 2015. *Inocybe griseorubida,* a new species of *Pseudosperma* clade from tropical India. Phytotaxa 221(2): 166–174. http://doi.org/10.11646/phytotaxa.221.2.6

Latha KPD, Manimohan P. 2016. Five new species of *Inocybe* (*Agaricales*) from tropical India. Mycologia 108: 110–122. https://doi.org/10.3852/14-358

Latha KPD, Manimohan P. 2017. Inocybes of Kerala. Spore Print Books, Calicut. 181 p.

Latha KPD, Manimohan P, Matheny PB. 2016. A new species of *Inocybe* representing the *Nothocybe* lineage. Phytotaxa 267: 40–50. https://doi.org/10.11646/phytotaxa.267.1.4

Matheny PB, Bougher NL. 2017. Fungi of Australia, *Inocybaceae*. Canberra & Melbourne.

Matheny PB, Aime MC, Henkel TW. 2003. New species of *Inocybe* from *Dicymbe* forests of Guyana. Mycological Research 107: 495–505. https://doi.org/10.1017/s0953756203007627

Matheny PB, Aime MC, Bougher NL, Buyck B, Desjardin DE, Horak E, Kropp BR, Lodge DJ, Trappe JM, Hibbett DS. 2009. Out of the Palaeotropics? Historical biogeography and diversification of the cosmopolitan mushroom family Inocybaceae. Journal of Biogeography 36: 577–592. https://doi.org/10.1111/j.1365-2699.2008.02055.x

Matheny PB, Aime MC, Smith ME, Henkel TW. 2012. New species and reports of *Inocybe* (*Agaricales*) from Guyana. Kurtziana 37: 23–39.

Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments Workshop (GCE) 14 Nov. 2010. Institute of Electrical and Electronics Engineers, New Orleans, LA. 8 p. https://doi.org/10.1109/GCE.2010.5676129

Naseer A, Khalid AN, Niazi AR. 2017. *Phylloporus brunneiceps* from Pakistan. Mycotaxon 132(3): 685–693. https://doi.org/10.5248/132.685

Naseer A, Khalid AN, Smith ME. 2018 ["2017"]. *Inocybe shawarensis* sp. nov. in the *Inosperma* clade from Pakistan. Mycotaxon 132(4): 909–918. https://doi.org/10.5248/132.909

Pradeep CK, Vrinda KB. 2007. Some noteworthy agarics from Western Ghats of Kerala. Journal of Mycopathological Research 1: 1–14.

Pradeep CK, Vrinda KB. 2010. Ectomycorrhizal fungal diversity in three different forest types and their association with endemic, indigenous and exotic species in the Western Ghats forests of Thiruvananthapuram district, Kerala. Journal of Mycopathological Research 48(2): 279–289.

Saba M, Ahmad I, Khalid AN. 2015. New reports of *Inocybe* from pine forests in Pakistan. Mycotaxon 130: 671–681. https://doi.org/10.5248/130.671

Shrestha BB. 2003. *Quercus semecarpifolia* Sm. in the Himalayan region: ecology, exploitation and threats. Himalaya Journal of Science 1: 126–128.

Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30: 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Tibpromma S, Hyde KD, Jeewon R. et al. 2017. Fungal diversity notes 491–602: taxonomic and phylogenetic contributions to fungal taxa. Fungal Diversity 83: 1–292. https://doi.org/10.1007/s13225-017-0378-0

Ullah Z, Jabeen S, Ahmad H, Khalid AN. 2018. *Inocybe pakistanensis,* a new species in section *Rimosae* s. str. from Pakistan. Phytotaxa 348(4): 279–288. http://doi.org/10.11646/phytotaxa.348.4.4

Vauras J, Larsson E. 2016 ("2015"). *Inocybe caprimulgi* and *I. lacunarum*, two new nodulose- spored species from Fennoscandia. Karstenia 55: 1–18. https://doi.org/10.29203/ka.2015.465

Vilgalys R, Hester M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology 172: 4238–4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990

Vrinda KB, Pradeep CK, Joseph AV, Abraham TK. 1996. A new *Inocybe* (*Cortinariaceae*) from Kerala State, India. Mycotaxon 57: 171–174.

Vrinda KB, Pradeep CK, Mathew S, Abraham TK. 1997. *Inocybe purpureoflavida* sp. nov. (*Cortinariaceae*) from Western Ghats of Kerala State, India. Mycotaxon 64: 1–6.

Watling R. 2001. An unusual *Inocybe* sp. from West Africa. Czech Mycology 52: 329–334.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, MA, Gelfand DH, Sninsky JJ, & White TJ, (Eds.) PCR Protocols: a guide to methods and applications. Academic Press, San Diego, California, 315–322. https://doi.org/10.1016/b978-0-12-372180-8.50042-1

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Sporidesmium horizontale sp. nov. from China

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Abstract—A new species, *Sporidesmium horizontale*, is described and illustrated from specimens collected on dead branches in China. This fungus is characterized by horizontal, obclavate, 4–5-septate, versicolored conidia with short rostra.

Key words—anamorphic fungi, hyphomycete, *Pleosporomycetidae*, taxonomy

Introduction

Sporidesmium was erected by Link (1809) and typified with *S. atrum* Link. Ellis (1971) defined the genus as having integrated, terminal, monoblastic conidiogenous cells with acrogenous, solitary, transversely septate or distoseptate conidia. Subramanian (1992) redefined the generic concept of *Sporidesmium* in a stricter sense as simple conidiophores, determinate or with irregular extending proliferations, and solitary, gangliar, acrogenous and euseptate conidia. Wu & Zhuang (2005) expanded the generic concepts to include fungi with typically lageniform, doliiform, or nodose, percurrently extending conidiophores. Since then 31 new species have been described in the genus, most of which were found on rotten wood, dead branches, or decaying

leaves of various plants (e.g., Ma & Zhang 2007; Ma & al. 2012; Xia & al. 2017; Yang & al. 2018; Zhang & al. 2008, 2017).

A new hyphomycete representing the genus of *Sporidesmium* was found during research on saprobic fungi in China. Specimens are deposited in the Herbarium of Department of Plant Pathology, Shandong Agricultural University, Taian, Shandong, China (HSAUP) and the Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China (HMAS).

Taxonomy

Sporidesmium horizontale L.G. Ma, C.C. Ai & X.G. Zhang, **sp. nov.** FIG. 1

MB 830543

Differs from other *Sporidesmium* species by its horizontal, obclavate, 4–5-septate, versicolored, and smooth conidia with a short, subhyaline rostrum.

TYPE: China, Yunnan Province, Xishuangbanna Tropical Botanical Garden, on dead stems of an unidentified broadleaf tree, 2 Nov. 2011, L.G. Ma (**Holotype**, HSAUP H2133; **isotype**, HMAS 146148).

ETYMOLOGY: *horizontale*, referring to the conidia with horizontal growth.

COLONIES on natural substrate effuse, brown to dark brown, hairy. Mycelium partly superficial, partly immersed in the substrate, composed of septate, pale brown, smooth, 3.0–4.5 µm diam. hyphae. CONIDIOPHORES macronematous, mononematous, unbranched, erect, straight to flexuous, cylindrical, smooth, thick-walled, brown to dark brown, 5–10-septate, 90–175 µm long, 6–13.5 µm diam. at the base and 3–4.5 µm diam. at the tapered apex. CONIDIOGENOUS CELLS holoblastic, monoblastic, brown, cylindrical, smooth, thick-walled, 5–6.5 × 17–21.5 µm. Conidial secession schizolytic. CONIDIA solitary, horizontal on the conidiophores, obclavate, thick-walled, brown, pale brown on the basal and upper cells, smooth, 4–5-euseptate, 41–59 × 16–18 µm, with a short, subhyaline rostrum at the apex, sometimes embedded in a drop of mucilage.

COMMENTS—*Sporidesmium horizontale* is unique in the genus by developing conidia in a horizontal orientation, an unusual phenomenon that cannot be explained at present. The species can also be separated from other *Sporidesmium* species by its horizontal, obclavate, 4–5-septate, and versicolored conidia with short, subhyaline rostra.

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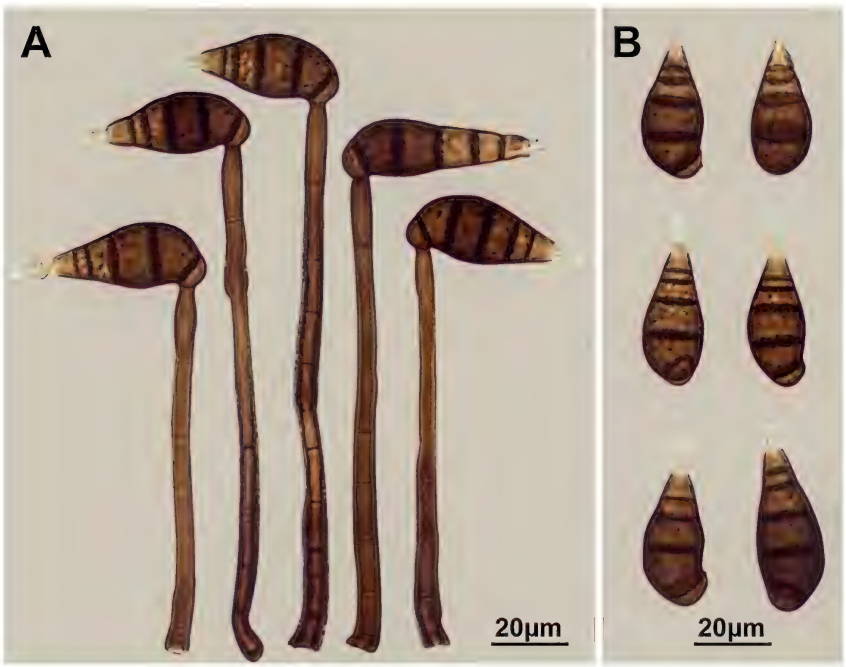

Fig. 1. *Sporidesmium horizontale* (holotype, HSAUP H2133).
A. Conidiophores, conidiogenous cells, conidia; B. Conidia.

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Literature cited

Ellis MB. 1971. Dematiaceous hyphomycetes. Commonwealth Mycological Institute, Kew, Surrey, England.

Link HF. 1809. Observationes in ordines plantarum naturales. Dissertatio I. Magazin der Gesellschaft Naturforschenden Freunde Berlin 3: 3–42.

Ma J, Zhang XG. 2007. Two new species of *Sporidesmium* from Yunnan, China. Mycotaxon 101: 73–76.

Ma J, Ma LG, Zhang YD, Xia JW, Zhang XG. 2012. New species and record of *Sporidesmium* from southern China. Mycotaxon 119: 17–25. https://doi.org/10.5248/119.17

Subramanian CV. 1992. A reassessment of *Sporidesmium* (hyphomycetes) and some related taxa. Proceedings of the Indian National Science Academy, Part B Biological Sciences 58: 179–189.

Wu WP, Zhuang WY. 2005. *Sporidesmium*, *Endophragmiella* and related genera from China. Fungal Diversity Research Series 15. 351 p.

Xia JW, Li HH, Zhang XG. 2017. Two new species of hyphomycetes from southern China. Mycosystema 36: 1483–1486. https://doi.org/10.13346/j.mycosystema.170140

Yang J, Maharachchikumbura SSN, Liu JK, Hyde KD, Jones EBG, Al-Sadi AM, Liu ZY. 2018. *Pseudostanjehughesia aquitropica* gen. et sp. nov. and *Sporidesmium* sensu lato species from freshwater habitats. Mycological Progress 17: 591–616. https://doi.org/10.1007/s11557-017-1339-4

Zhang K, Ma J, Zhang XG. 2008. Taxonomic studies of *Sporidesmium* from Hainan, China. Mycotaxon 104: 165–169.

Zhang H, Dong W, Hyde KD, Maharachchikumbura SSN, Hongsanan S, Bhat DJ, Al-Sadi AM, Zhang D. 2017. Towards a natural classification of *Annulatascaceae*-like taxa: introducing *Atractosporales* ord. nov. and six new families. Fungal Diversity 85: 75–110. https://doi.org/10.1007/s13225-017-0387-z

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Sporidesmiella corniformis sp. nov. from China

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Abstract—A new species, *Sporidesmiella corniformis*, is described and illustrated from specimens collected on decaying branches in China. This fungus is characterized by clavate to cuneiform, 2-distoseptate, pale brown conidia with 4–8 short horns.

Key words—anamorphic fungi, hyphomycete, *Melanommataceae,* taxonomy

Introduction

Numerous microfungi collected in tropical and subtropical forests in China have recently been reported (Ma & al. 2016a, b). In our continual mycological surveys on wood-inhabiting fungi in China, an anamorphic fungus with morphological features typical of *Sporidesmiella* Kirk (1982) was collected. However, this fungus is morphologically different from the currently described *Sporidesmiella* species and is therefore proposed as new.

Specimens are deposited in the Herbarium of the Department of Plant Pathology, Shandong Agricultural University, Taian, Shandong, China (HSAUP) and the Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China (HMAS).

Materials & methods

Samples of partially decomposed woody debris collected were placed in separate polyethylene bags for transport to the laboratory. Samples were incubated, examined, and processed periodically following the methods described in Ma & al. (2012a). Conidia and conidiophores were measured and photographed using a DP 27 camera attached to a Olympus BX 53 microscope equipped with a 60× oil immersion objective. Then photographic plate was prepared using Adobe Photoshop CS5.

Taxonomy

Sporidesmiella corniformis L.G. Ma, C.C. Ai & X.G. Zhang, **sp. nov.** Fig. 1

MB 830544

Differs from *Sporidesmiella cornuta* by its bigger, 2-distoseptate conidia with much shorter, more numerous horns; and from *S. coronata* by its 2-distoseptate, wider conidia with longer, more numerous horns.

Type: China, Yunnan Province, Xishuangbanna National Forest Park, on dead branches of an unidentified broadleaf tree, 12 Oct. 2011, L.G. Ma (**Holotype**, HSAUP H2058; **isotype**, HMAS 146151).

Etymology: *corniformis*, referring to the conidial shape.

Colonies on natural substrate effuse, brown to dark brown, hairy. Mycelium partly superficial, partly immersed in the substrate, composed of septate, pale brown, smooth, 1.5–3.0 µm diam. hyphae. Conidiophores macronematous, mononematous, simple, unbranched, erect, straight to slightly flexuous, cylindrical, smooth, thick-walled, brown to dark brown, 4–11-septate, 55–175 × 3.5–5 µm. Conidiogenous cells integrated, terminal, holoblastic, monoblastic, brown, cylindrical, smooth, thick-walled, percurrently extended, with 0–5 cylindrical extensions towards the apex. Conidial secession schizolytic. Conidia acrogenous, solitary, clavate to cuneiform, thick-walled, pale brown, smooth, 2-distoseptate, 21–35 µm long, 11.5–17.0 µm wide at the apex, 2.5–3.5 µm wide at the truncate base, apex with 4–8 short horns 2.0–7.5 µm long; cell lumina reduced, less so in the basal cell, the basal cell darker and basal septum convex and deeply pigmented.

Comments—*Sporidesmiella* was erected by Kirk (1982) with *S. claviformis* P.M. Kirk as the type species. The genus is characterized by acrogenous, solitary, distoseptate conidia seceding schizolytically from integrated, terminal, monoblastic, percurrent, or rarely sympodially extended conidiogenous cells on macronematous, mononematous, solitary conidiophores. Although it shares some resemblance to *Coronospora* (Ellis 1971), the latter genus is distinguished by polyblastic, sympodial, cicatrized conidiogenous cells bearing euseptate conidia. Both Yanna & al. (2001) and Ma & al. (2012b) reviewed *Sporidesmiella*

Fig. 1. *Sporidesmiella corniformis* (holotype, HSAUP H2058). A. Conidiophores, conidiogenous cells and conidia; B. Conidiophore with conidiogenous cell; C. Conidia.

and provided a key to the accepted species. Hitherto, 38 species have been discovered, of which 12 were described from China.

Sporidesmiella corniformis is most similar to *S. cornuta* Kuthub. & Nawawi and *S. coronata* (B. Sutton) P.M. Kirk. *Sporidesmiella cornuta* differs by its conidia that are 2–3-distoseptate, cylindrical to cuneiform, 24–29 µm long and 8–13 µm diam. towards the apex and 4–5 µm diam. at the base and with (2–)3(–4) acuminate horns that are 10–24 µm long (Kirk 1982). *Sporidesmiella*

coronata can be separated by its conidia, which are 2–3-distoseptate, 6–7.5 µm diam. towards the apex and with 4 short (≤4 µm) horns (Kuthubutheen & Nawawi 1993).

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The authors express gratitude to Dr. Rafael F. Castañeda-Ruiz (Alejandro de Humboldt, INIFAT, Cuba) and Dr. De-Wei Li (Connecticut Agricultural Experiment Station Valley Laboratory, USA) for serving as pre-submission reviewers and for their valuable comments and suggestions. This project was supported by National Key R&D Program of China (2016YFD0300700), Young Talents Training Program of Shandong Academy of Agricultural Sciences (CXGC2018E04), National Natural Science Foundation of China (31400019, 31230001, 31093440), the Ministry of Science and Technology of the People's Republic of China (2006FY120100), and Natural Science Foundation of Shandong Province (BS2015SW020).

Literature cited

Ellis MB. 1971. Dematiaceous hyphomycetes. X. Mycological Papers 125. 30 p.

Kirk PM. 1982. New or interesting microfungi. VI. *Sporidesmiella* gen. nov. (hyphomycetes). Transactions of the British Mycological Society 79: 479–489. https://doi.org/10.1016/s0007-1536(82)80040-5

Kuthubutheen AJ, Nawawi A. 1993. Three new and several interesting species of *Sporidesmiella* from submerged litter in Malaysia. Mycological Research 97: 1305–1314. https://doi.org/10.1016/s0953-7562(09)80162-7

Ma LG, Ma J, Zhang YD, Castañeda-Ruiz RF, Zhang XG. 2012a. New species and records of *Heteroconium* (anamorphic fungi) from southern China. Mycoscience 53: 466–470. https://doi.org/10.1007/s10267-012-0190-3

Ma J, Zhang YD, Ma LG, Castañeda-Ruiz RF, Zhang XG. 2012b. Three new species of *Sporidesmiella* from southern China. Mycoscience 53: 187–193. https://doi.org/10.1007/s10267-011-0152-1

Ma J, Zhang K, Zhang XG, Castañeda-Ruiz RF. 2016a. Three new species of *Spadicoides* from Lushan Mountain, China. Mycological Progress 15: 43 [8 p.]. https://doi.org/10.1007/s11557-016-1185-9

Ma YR, Xia JW, Gao JM, Li XY, Castañeda-Ruiz RF, Zhang XG, Li Zhuang. 2016b. *Atrokylindriopsis*, a new genus of hyphomycetes from Hainan, China, with relationship to *Chaetothyriales*. Mycological Progress 14: 77 [5 p.]. https://doi.org/10.1007/s11557-015-1071-x

Yanna, Ho WH, Hyde KD, McKenzie EHC. 2001. *Sporidesmiella oraniopsis*, a new species of dematiaceous hyphomycete from North Queensland, Australia and synopsis of the genus. Fungal Diversity 8: 183–190.

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New records of *Amandinea* and *Buellia* from China

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Abstract—Our research has revealed two species of *Amandinea* (*A. occidentalis* and *A. polyspora*) and two species of *Buellia* (*B. morsina* and *B. penichra*), which we report for the first time from China. Descriptions of morphological and chemical characters are provided with the known distribution of each species.

Key words—*Caliciaceae*, *Caliciales*, corticolous, lichen-forming fungi, taxonomy

Introduction

Amandinea M. Choisy ex Scheid. & M. Mayrhofer and *Buellia* De Not. (*Caliciaceae*, *Caliciales*, *Lecanoromycetes*, *Ascomycota*) are closely related genera characterized by a crustose thallus, apothecial ascomata, (4–)8(–many)-spored asci, and brown 1-septate or (sub)muriform ascospores, with or without oil droplets in the hymenium (Foucard & al. 2002, Coppins & al. 2009). *Amandinea* can best be distinguished by the filiform conidia, ascospores with or without inner wall thickenings and clearly ornamented outer walls, and often lacking secondary metabolites detected by TLC, while species of *Buellia* s. lat. have bacilliform conidia and usually contain secondary compounds detectable by TLC (Coppins & al. 2009, Elix & Kantvilas 2013a,b, Giralt & al. 2015). *Amandinea* is represented by 81 species and *Buellia* 454 species worldwide (Giralt & Nordin 2002; Kirk & al. 2008; Şenkardeşler 2010; Giralt & al. 2011, 2015; Lumbsch & al. 2011;

[#] Xiao Zhang & Zun-Tian Zhao contributed equally to this research.

Giralt & van den Boom 2012; Fryday & Arcadia 2012; Tønsberg & al. 2012, Elix & Kantvilas 2013a,b, 2016a,b; Kondratyuk & al. 2013, 2015, 2016; Elix & al. 2015, 2017a,b, 2018; Blaha & al. 2016; Mayrhofer & al. 2016; Elix 2017a,b; Elix & Mayrhofer 2017, 2018). In China, only one *Amandinea* species and 53 *Buellia* species have been reported (Wei 1991, Watanuki & al. 2017, Wang & al. 2018).

This paper contributes to the knowledge of *Amandinea* and *Buellia* in China and affords accurate data for the Lichen Flora of China project. In this study we have identified four species new to the country: *Amandinea occidentalis*, *A. polyspora*, *Buellia morsina*, and *B. penichra*.

Materials & methods

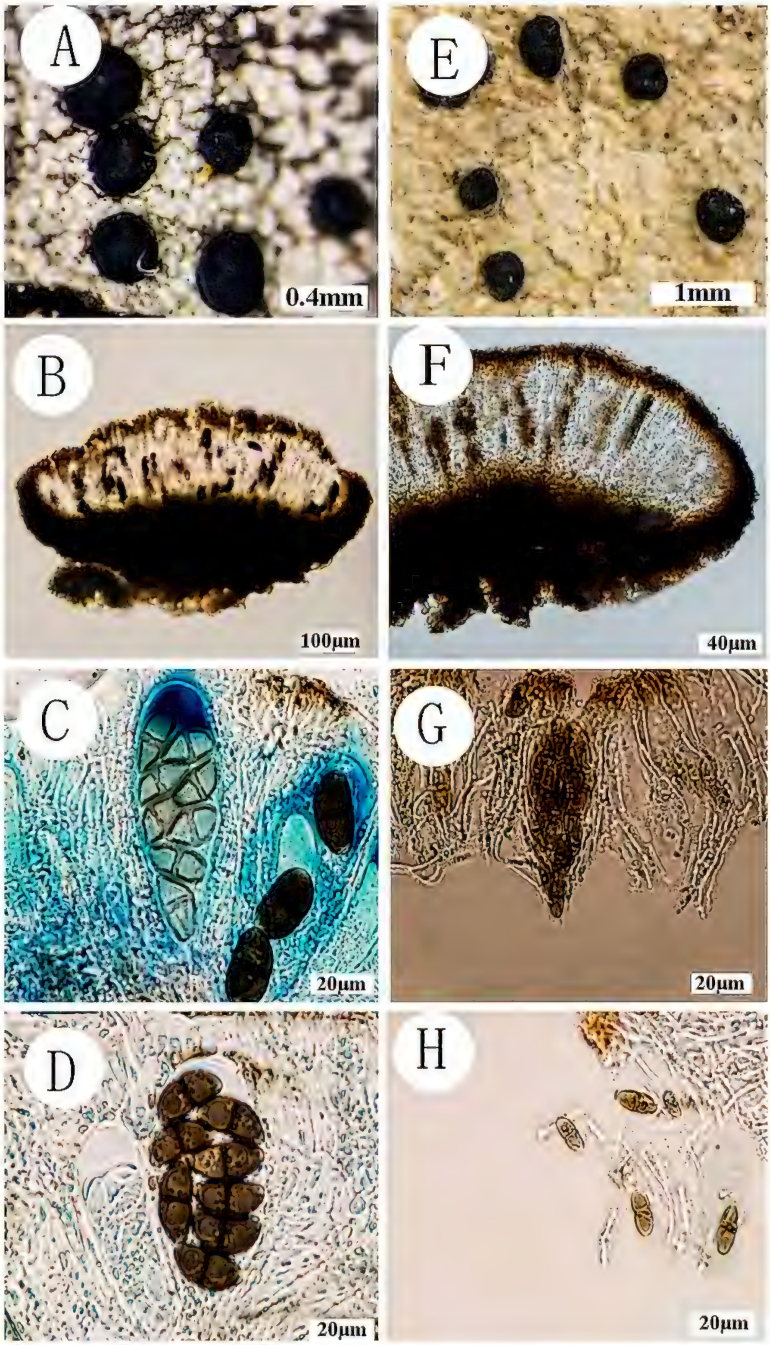
The specimens studied are preserved in the Lichen Section of Botanical Herbarium, Shandong Normal University, Jinan, China (SDNU). The specimens were examined morphologically and anatomically under a COIC XTL7045B2 stereo-microscope and a Olympus CX41 polarizing microscope. We tested the thallus and medulla of each specimen with K (10% aqueous KOH solution), C (aqueous ClNaO solution), and I (Lugol's iodine) for identification. Lichen substances were identified using standardized thin-layer chromatography techniques (TLC) with system C (Orange & al. 2010). The lichens were photographed using Olympus SZX16 and BX61 microscope with a DP72 lens.

Taxonomy

Amandinea occidentalis Elix & Kantvilas,
Australas. Lichenol. 72: 9 (2013) FIG. 1A–D

Thallus crustose, smooth, thin, areolate, pale greyish to white, esorediate. Prothallus present, but not apparent. Medulla white, I–. Apothecia black, 0.2–0.5 mm diam., sessile, epruinose, lecideine with a well developed margin, weakly concave at first, then plane to convex, scattered or crowded. Proper exciple dark brown, 50–70 µm wide; epihymenium olive-brown, K–; hymenium hyaline, inspersed with oil droplets; paraphyses simple; hypothecium dark brown, K–. Asci (4–)8-spored, *Bacidia*-type. Ascospores soon olive-brown, 1-septate, at first of the *Physconia*-type, then of the *Buellia*-type, broadly fusiform to ellipsoid, 23–28 × 9–11 µm, not constricted at the septum. Pycnidia not observed.

FIG. 1 ***Amandinea occidentalis*** (Wang 20181068, SDNU). A: Thallus with apothecia; B: Apothecium section; C: Ascus containing *Physconia*-type ascospores; D: Ascus containing *Buellia*-type ascospores. ***Amandinea polyspora*** (Cheng 20120079, SDNU). E: Thallus with apothecia; F: Apothecium section, without oil droplets in hymenium G: Ascus and ascospores; H: Ascospores.


A
0.4mm
E
1mm
B
100μm
F
40μm
C
20μm
G
20μm
D
20μm
H
20μm

Chemistry—Thallus K–, C–. No chemical substances detected by TLC.

Specimen examined: **CHINA. Yunnan, Shangri-La,** Mt. Tianbao, alt. 3700 m, on bark, 17 Aug. 2018, Chunxiao Wang 20181068 (SDNU).

Distribution—Australia (Western Australia, New South Wales) (Elix & Kantvilas 2013a; McCarthy 2018). New to China.

Comments—Our specimens are similar to previously published descriptions, except that the holotype has larger (0.2–1.2 mm) apothecia and usually 4-spored asci (Elix & Kantvilas 2013a). *Amandinea occidentalis* is characterized by the areolate thallus, *Physconia*-type to *Buellia*-type ascospores, a hymenium inspersed with oil droplets, and the absence of lichen substances. This species morphologically resembles *A. lignicola* var. *australis* Elix & Kantvilas, which is distinguished by often having a subsquamulose thallus, 8-spored asci with smaller ascospores (11–20 × 5–8 μm), and a non-inspersed hymenium (Elix & Kantvilas 2013a).

Amandinea polyspora (Willey) E. Lay & P.F. May,
Bryologist 100(2): 164 (1997) Fig. 1e–h

Thallus corticate, smooth, thin, pale brown to yellowish brown, esorediate; medulla white, I–. Apothecia lecideine, black, 0.2–0.5 mm diam., sessile, epruinose, flat to convex, margin present at first but soon excluded. Proper exciple narrow, dark brown, 20–30 μm wide; epihymenium brown, pigmentation continuous with the outer exciple; hymenium hyaline, not inspersed with oil droplets; paraphyses simple to moderately branched, swollen at upper part, with a brown pigmented cap; hypothecium reddish brown. Asci *Bacidia*-type, mostly 16-spored, occasionally 32-spored. Ascospores soon brown, 1-septate, *Buellia*-type, oblong to ellipsoid, not constricted, 10–13(–15) × 4–5 μm. Pycnidia not seen.

Chemistry—Thallus K+ yellow, C–. Atranorin (trace) detected by TLC.

Specimen examined: **CHINA. Jilin, Helong,** Mt. Zengfeng, alt. 1600 m, on bark, 19 Aug. 2011, Yuliang Cheng 20120079 (SDNU).

Distribution—*Amandinea polyspora* has been reported from Korea (Kondratyuk & al. 2013) and from western, eastern, and southeastern USA (Bungartz & al. 2007). New to China.

Comments—Our specimens are similar to the protologue descriptions (Bungartz & al. 2007). *Amandinea polyspora* is characterized by the small ascospores and the polysporous asci. *Buellia schaereri* De Not. has similarly small ascospores but differs in its consistently 8-spored asci and bacilliform

conidia, unlike the filiform conidia characteristic of *A. polyspora* (Bungartz & al. 2007).

Buellia morsina A. Nordin, Symb. Bot. Upsal. 33(1): 74 (2000) FIG. 2A–C

Thallus crustose, smooth, rimose to areolate, brownish to grey, esorediate. Prothallus absent. Medulla white, I–. Apothecia lecideine, 0.7–1.5 mm diam.; sessile, disk black, epruinose, concave in young then flat; margin black, obvious in young apothecia. Proper exciple dark brown, 50–100 µm wide; epihymenium dark brown, K–, C–. hymenium colorless, 80–125 µm, not inspersed with oil droplets. Hypothecium dark brown. Asci 8-spored; ascospores soon brown, submuriform to muriform, 20–30 × 10–13 µm. Pycnidia not observed.

CHEMISTRY—Thallus K+ yellow turning red. Norstictic acid, atranorin and connorstictic acid (trace) detected by TLC.

SPECIMEN EXAMINED: **CHINA. YUNNAN, Luquan**, Mt. Jiaozi, alt. 3600 m, on bark, 4 Nov. 2012, Hui Zhang 20129415 (SDNU).

DISTRIBUTION—*Buellia morsina* has previously been reported from Mexico (Bungartz & al. 2007). New to China.

COMMENTS—Our specimens are similar to previously published descriptions, except that the protologue describes smaller apothecia (0.2–1.2 mm) and the presence of a black prothallus (Bungartz & al. 2007). *Buellia morsina* is characterized by its submuriform to muriform ascospores and the presence of norstictic acid, atranorin, and connorstictic acid (trace). Its thallus morphology, secondary chemistry, and exciple anatomy are similar to *B. lauri-cassiae* (Fée) Müll. Arg., which is distinguished by its smaller, 3-septate spores (Nordin 2000, Giralt & Nordin 2002, Bungartz & al. 2007).

Buellia penichra (Tuck.) Hasse, Contr. U.S. Natl. Herb. 17: 122 (1913) FIG. 2D–F

Thallus corticate, smooth, greyish white, rimose to dispersed areolate. Prothallus black. Medulla white, I–. Apothecia lecideine, black, round, 0.4–0.8 mm diam., sessile, dispersed, flat at first soon convex, disc epruinose; margin black, distinct in young apothecia, later excluded. Proper exciple brown, pigmentation continuous with the outer exciple; epihymenium brown to dark green; hymenium hyaline, inspersed with oil droplets, paraphyses simple with distinct apical cap; hypothecium dark brown to dark green. Asci 8-spored, ascospores submuriform, pale green then brown, broadly fusiform to ellipsoid, 25–28 × 10–13 µm. Pycnidia not observed.

CHEMISTRY—Thallus K+ yellow, C–. Atranorin detected by TLC.

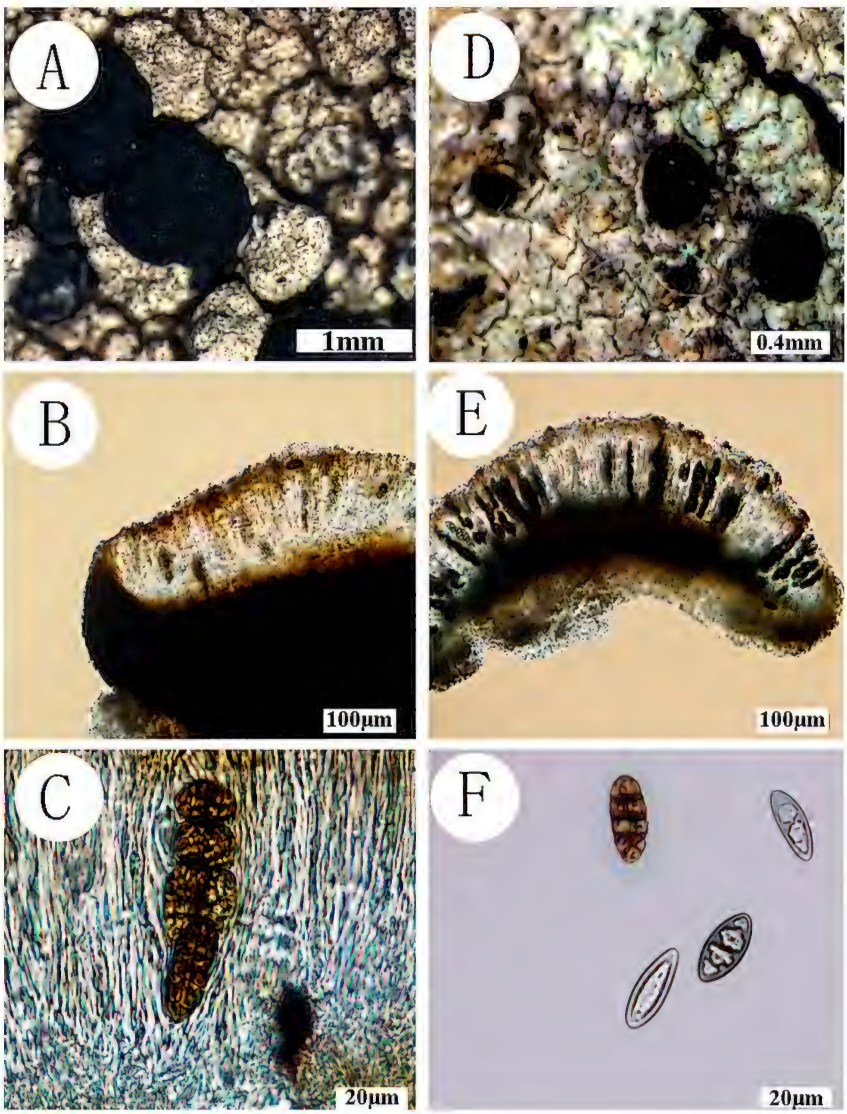


Fig. 2 ***Buellia morsina*** (Zhang 20129415, SDNU). A: Thallus with apothecia; B: Apothecium section; C: Ascus and ascospores. ***Buellia penichra*** (Wang 20181108, SDNU). D: Thallus with apothecia; E: Apothecium section; F: Ascospores, showing stages of development.

Specimens examined: **CHINA. Yunnan, Shangri-La**, Mt. Tianbao, alt. 3790 m, on bark, 17 Aug. 2018, Chunxiao Wang 20181131 (SDNU); alt. 3845 m, on bark, 17 Aug. 2018, Chunxiao Wang 20181108 (SDNU).

Distribution—Western North America (Rocky Mountains) (Bungartz & al. 2007). New to China.

Comments—The protologue describes *Buellia penichra* as containing atranorin and sometimes placodiolic acid (Bungartz & al. 2007), but our specimens contain only atranorin. This species is characterized by the submuriform spores lacking a perispore and presence of atranorin. *Buellia penichra* might be confused with *B. muriformis* A. Nordin & Tønsberg on the basis of its ascospores lacking a perispore, but *B. muriformis* differs in its secondary chemistry (containing placodiolic acid, atranorin, isousnic acid, brialmontin 1 and brialmontin 2) and larger (25–39 × 13–18 μm) ascospores (Nordin 1999).

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Literature cited

Blaha J, Mayrhofer H, Elix JA. 2016. Five new saxicolous species of *Amandinea* (*Ascomycota, Physciaceae*) from New Zealand and southern Australia. Australasian Lichenology 79: 35–49.

Bungartz F, Nordin A, Grube U. 2007. *Buellia* De Not. 113–179, in: TH Nash III & al. (eds). Lichen Flora of the Greater Sonoran Desert Region, vol. 3. Tempe, Lichens Unlimited, Arizona State University.

Coppins BJ, Scheidegger C, Aptroot A. 2009. *Buellia* de Not. (1846). 238–239, in: CW Smith & al. (eds). The lichens of Great Britain and Ireland. London, British Lichen Society. https://doi.org/10.1017/S0024282909990521

Elix JA. 2017a. Two new species and new records of buellioid lichens (*Caliciaceae*, *Ascomycota*) from Macquarie Island. Australasian Lichenology 81: 6–11.

Elix JA. 2017b. Three new species and eight new records of saxicolous buellioid lichens (*Caliciaceae*, *Ascomycota*) from New Zealand's Subantarctic Islands. Australasian Lichenology 81: 68–78

Elix JA, Kantvilas G. 2013a. New taxa and new records of *Amandinea* (*Physciaceae*, *Ascomycota*) in Australia. Australasian Lichenology 72: 3–19.

Elix JA, Kantvilas G. 2013b. New taxa and new records of *Buellia* sensu lato (*Physciaceae*, *Ascomycota*) in Australia. Australasian Lichenology 73: 24–44.

Elix JA, Kantvilas G. 2016a. *Amandinea coniops* (*Physciaceae, Ascomycota*) and its mimics in Tasmania and New Zealand. Australasian Lichenology 78: 22–31.

Elix JA, Kantvilas G. 2016b. New species and new records of buellioid lichens (*Ascomycota, Physciaceae*) in Tasmania. Australasian Lichenology 79: 26–34.

Elix JA, Mayrhofer H. 2017. New species and new records of buellioid lichens (*Physciaceae*, *Ascomycota*) from New Zealand. Telopea 20: 75–84. https://doi.org/10.7751/telopea11334.

Elix JA, Mayrhofer H. 2018. Three new species and ten new records of buellioid lichens (*Ascomycota*, *Caliciaceae*) from New Zealand. Australasian Lichenology 82: 68–79.

Elix JA, Malcolm WM, Knight A. 2015. New records and new combinations of buellioid lichens (*Physciaceae*, *Ascomycota*) from New Zealand. Australasian Lichenology 77: 36–41.

Elix JA, Kantvilas G, McCarthy PM. 2017a. Thirteen new species and a key to buellioid lichens (*Caliciaceae*, *Ascomycota*) in Australia. Australasian Lichenology 81: 26–67.

Elix JA, Mayrhofer H, McCarthy PM. 2017b. New species and a new record of buellioid lichens (*Physciaceae*, *Ascomycota*) from Australia. Australasian Lichenology 80: 28–37.

Elix JA, Mayrhofer H, Rodriguez JM. 2018. Two new species, a new combination and four new records of saxicolous buellioid lichens (*Ascomycota*, *Caliciaceae*) from southern South America. Australasian Lichenology 83: 3–13.

Foucard T, Mayrhofer H, Moberg R, Nordin A. 2002. Nordic lichen flora, vol. 2. Svenska Botaniska Föreningen.

Fryday AM, Arcadia L. 2012. Typification and revised basionym for *Fuscidea lygaea*, and a new name for *Amandinea lecideina*. Graphis Scripta 24(2): 40–44.

Giralt M, Nordin A. 2002. *Buellia triseptata* in the Iberian Peninsula. Lichenologist 34(2): 89–94. https://doi.org/10.1006/lich.2002.0375

Giralt M, van den Boom PPG. 2012. Checklist and three new species of lichens and lichenicolous fungi of the Algarve (Portugal). Sydowia 64(2): 149–208.

Giralt M, van den Boom PPG, Elix JA. 2011. *Amandinea myrticola*, a new corticolous species from Portugal. Lichenologist 43(3): 193–197. https://doi.org/10.1017/S0024282911000120

Giralt M, van den Boom PPG, Elix JA. 2015. *Amandinea lobarica*, a new corticolous species from Guatemala, with notes on some additional *Amandinea* taxa. Lichenologist 47(4): 257–268. https://doi.org/10.1017/S0024282915000158

Kirk PM, Cannon PF, Minter DW, Stalpers JA (eds). 2008. Dictionary of the fungi, 10th edition. CAB International, Wallingford.

Kondratyuk SY, Lőkös L, Tschabanenko S, Moniri MH, Farkas E, Wang SY, Oh SO, Hur JS. 2013. New and noteworthy lichen-forming and lichenicolous fungi. Acta Botanica Hungarica 55(3–4): 275–349. https://doi.org/10.1556/abot.55.2013.3-4.9

Kondratyuk SY, Lőkös L, Farkas E, Oh SO, Hur, JS. 2015. New and noteworthy lichen-forming and lichenicolous fungi 3. Acta Botanica Hungarica 57(3–4): 345–382. https://doi.org/10.1556/034.57.2015.3-4.7

Kondratyuk SY, Lőkös L, Halda JP, Upreti DK, Mishra GK, Moniri MH, Farkas E, Park JS, Lee BG, Liu D, Woo JJ, Jayalal RGU, Oh SO, Hur, JS. 2016. New and noteworthy lichen-forming and lichenicolous fungi: 5. Acta Botanica Hungarica 58(3–4): 319–396. https://doi.org/10.1556/abot.58.2016.3-4.7

Lumbsch T, Ahti T, Altermann S, Arup U & al. 2011. One hundred new species of lichenized fungi: a signature of undiscovered global diversity. Phytotaxa 18(1). 127 p.

Mayrhofer H, Ropin K, Elix JA. 2016. Two new corticolous species of *Amandinea* (*Ascomycota*, *Physciaceae*) from New Zealand. Australasian Lichenology 78: 11–12.

McCarthy PM. 2018. Checklist of the lichens of Australia and its island territories. Australian Biological Resources Study, Canberra. Version 17 May 2018. https://www.anbg.gov.au/abrs/lichenlist/introduction.html

Nordin A. 1999. *Buellia* species with pluriseptate spores: new and unrecorded species in North America. Bryologist 102(2): 249–264. https://doi.org/10.2307/3244364

Nordin A. 2000. Taxonomy and phylogeny of *Buellia* species with pluriseptate spores (*Lecanorales*, *Ascomycotina*). Symbolae Botanicae Upsalienses 33(1). 117 p.

Orange A, James PW, White FJ. 2010. Microchemical methods for the identification of lichens. 2nd edition. London, British Lichen Society.

Şenkardeşler A. 2010. Additions and corrections of types in the genus *Buellia* s. lat. (*Physciaceae*) described by J. Steiner. Lichenologist 42(4): 439–448. https://doi.org/10.1017/S0024282910000241

Tønsberg T, Nordin A, Tibell L. 2012. *Amandinea lignicola*, a new species from the Pacific coast of North America. Graphis Scripta 24(2): 60–64.

Wang QD, Liu FY, Wu XH, Zhao X, Jia ZF. 2018. *Buellia taishanensis* sp. nov. and new *Buellia* records from Mt. Tai, China. Mycosystema 133: 165–172. https://doi.org/10.5248/133.165

Watanuki O, Hara K, Harada H, Komine M, Fuji S. 2017. *Buellia numerosa* and *B. subnumerosa*, two new species of the lichen genus *Buellia* (*Caliciaceae*) from Japan. Bryologist 120(1): 25–36. https://doi.org/10.1639/0007-2745-120.1.025

Wei JC. 1991. An enumeration of lichens in China. Beijing, International Academic Publishers. 278 p.

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Introduction of subfamily names for four clades in *Cladoniaceae* and *Peltigeraceae* (*Lecanoromycetes*)

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Abstract—In a recent proposal for a classification of orders and families in *Lecanoromycetidae* and *Ostropomycetidae* based on a temporal approach, *Squamarinaceae* and *Stereocaulaceae* were synonymized with *Cladoniaceae*, and *Lobariaceae* and *Nephromataceae* with *Peltigeraceae*. Since these four synonymized families represent easily recognized, monophyletic lineages and are well-established names, we here propose to accept them at subfamilial rank, as *Lobarioideae*, *Nephromatoideae*, *Squamarinoideae*, and *Stereocauloideae*.

Key words—classification, *Lecanorales*, *Peltigerales*, phylogeny, taxonomy

Introduction

Recently we proposed a revised classification of orders and families in the two major subclasses of *Lecanoromycetes*, *Lecanoromycetidae* and *Ostropomycetidae* (Kraichak & al. 2018) based on a temporal banding approach (Avise & Johns 1999, Kraichak & al. 2017). This classification proposal differed at the family level from other recently proposed classifications (Jaklitsch & al. 2016; Lücking & al. 2016, 2017; Lumbsch & Huhndorf 2010; Wijayawardene & al. 2018) in accepting some clades at family level (such as *Diploschistaceae*, *Thelotremataceae*, and *Varicellariaceae*) while synonymizing other clades previously accepted as families within older families. The latter clades represented monophyletic lineages but were shown to have diverged more recently than 111–235 Mya,

which was the temporal band identified for the family rank. Four of the families synonymized in Kraichak & al. (2018) have been widely used names, and, hence, we suggest they should be accepted at subfamily rank. This allows recognition of these well-established and monophyletic clades while avoiding confusion due to incomparable ranks of clades.

Below we formally propose new subfamilies for the previously accepted families *Lobariaceae*, *Nephromataceae*, *Squamarinaceae*, and *Stereocaulaceae*. With the description of these subfamilies, *Cladonioideae* will become an available name for *Cladoniaceae* s.str. (i.e., excluding *Squamarinoideae* and *Stereocauloideae*) and *Peltigeroideae* for *Peltigeraceae* s.str. (i.e., excluding *Lobarioideae* and *Nephromatoideae*).

Taxonomy

Cladoniaceae Zenker, in Goebel & Kunze, Pharmac. Waarenk. 1(3): 124 (1827).

Squamarinoideae Lumbsch & S.D. Leav., **stat. nov.**
MB 830561
Basionym: *Squamarinaceae* Hafellner, Beih. Nova Hedwigia 79: 342 (1984).

Stereocauloideae Lumbsch & S.D. Leav., **stat. nov.**
MB 830562
Basionym: *Stereocaulaceae* Chevall., Fl. Gén. Env. Paris 1: 596 (1826).

Peltigeraceae Dumort., Comment. Bot.: 68 (1822).

Lobarioideae Lumbsch & S.D. Leav., **stat. nov.**
MB 830563
Basionym: *Lobariaceae* Chevall., Fl. Gén. Env. Paris 1: 609 (1826).

Nephromatoideae Lumbsch & S.D. Leav., **stat. nov.**
MB 830564
Basionym: *Nephromataceae* Wetmore ex J.C. David & D. Hawksw., Syst. Ascom. 10: 15 (1991).

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Literature cited

Avise JC, Johns GC. 1999. Proposal for a standardized temporal scheme of biological classification for extant species. Proceedings of the National Academy of Science of the United States of America 96: 7358–7363. https://doi.org/10.1073/pnas.96.13.7358

Jaklitsch WM, Baral HO, Lücking R, Lumbsch HT. 2016. Syllabus of plant families — A. Engler's Syllabus der Pflanzenfamilien, Part 1/2 *Ascomycota*. Gebr. Borntraeger Verlagsbuchhandlung. 322 p.

Kraichak E, Crespo A, Divakar PK, Leavitt SD, Lumbsch HT. 2017. A temporal banding approach for consistent taxonomic ranking above the species level. Scientific Reports 7:2297 [7 p.]. https://doi.org/10.1038/s41598-017-02477-7

Kraichak E, Huang JP, Nelsen M, Leavitt SD, Lumbsch HT. 2018. A revised classification of orders and families in the two major subclasses of *Lecanoromycetes* (*Ascomycota*) based on a temporal approach. Botanical Journal of the Linnean Society 188: 233–249. https://doi.org/10.1093/botlinnean/boy060

Lücking R, Hodkinson BP, Leavitt SD. 2016. The 2016 classification of lichenized fungi in the *Ascomycota* and *Basidiomycota* – approaching one thousand genera. Bryologist 119: 361–416. https://doi.org/10.1639/0007-2745-119.4.361

Lücking R, Hodkinson BP, Leavitt SD. 2017. Corrections and amendments to the 2016 classification of lichenized fungi in the *Ascomycota* and *Basidiomycota*. Bryologist 120: 58–69. https://doi.org/10.1639/0007-2745-120.1.058

Lumbsch HT, Huhndorf SM. 2010. Myconet volume 14. Part one. Outline of *Ascomycota*—2009. Fieldiana Life and Earth Sciences 1: 1–42. https://doi.org/10.3158/1557.1

Wijayawardene NN, Hyde KD, Lumbsch HT, Liu JK, Maharachchikumbura SSN, Ekanayaka AH, Tian Q, Phookamsak R. 2018. Outline of *Ascomycota*: 2017. Fungal Diversity 88: 167–263. https://doi.org/10.1007/s13225-018-0394-8

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Cacumisporium fusiforme sp. nov. from Jiangxi, China

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Abstract—A new species, *Cacumisporium fusiforme*, is described and illustrated from a specimen collected on dead branches of an unidentified broadleaf tree in Jiangxi, China. The fungus differs from other described *Cacumisporium* species in its conidial shape and size. A key to *Cacumisporium* species is provided.

Key words—asexual fungi, hyphomycetes, saprobes, taxonomy

Introduction

Cacumisporium Preuss, was established by Preuss (1851) for a single species *C. tenebrosum* Preuss. The genus is diagnosed by differentiated, unbranched, septate conidiophores and solitary, acropleurogenous, euseptate, pigmented conidia that secede schizolytically from polyblastic, integrated, terminal, flattened or inconspicuous denticulate conidiogenous cells after several holoblastic sympodial elongations on enteroblastic percurrent regenerated portions of the conidiophores (Goos 1969, Tsui & al. 2001, Castañeda-Ruíz & al. 2007a,b, Wongsawas & al. 2009, Seifert & al. 2011). Saccardo

(1877) recombined *C. tenebrosum* as *Acrothecium tenebrosum* (Preuss) Sacc., but *Acrothecium* is now treated as an unaccepted genus based on an unidentifiable type (Hughes 1958, Seifert & al. 2011). In modern literature, *Cacumisporium tenebrosum* is treated as a junior synonym of *C. capitulatum* (Corda) S. Hughes [≡ *Helminthosporium capitulatum* Corda; ≡ *Acrothecium capitulatum* (Corda) Ferraris] (Mangenot 1953, Hughes 1958, Sutton 1973, Seifert & al. 2011). Eight other species have been published in *Cacumisporium* (Mercado-Sierra & Castañeda-Ruíz 1987, Castañeda-Ruíz & Kendrick 1991, Kirk 1992, Tsui & al. 2001, Castañeda-Ruíz & al. 2007a,b, Wongsawas & al. 2009, Rambelli & Raimondo 2014). However, "*C. oceanicum*" Rambelli is invalid (Index Fungorum 2018), and *C. curvularioides* R.F. Castañeda & W.B. Kendr. has been synonymised with *C. pleuroconidiophorum* (Davydkina & Melnik) R.F. Castañeda & al. (Castañeda-Ruíz & al. 2007a), leaving *Cacumisporium* currently with seven valid taxa. The criteria used to delimit species are based primarily on conidial shape, size, septation, and pigmentation (Kirk 1992; Tsui & al. 2001; Castañeda-Ruíz & al. 2007a,b; Wongsawas & al. 2009). A key to *Cacumisporium* species was provided by Castañeda-Ruíz & al. (2007b).

The forests of Jiangxi have a rich fungal diversity, and many anamorphic fungi have been discovered there (Ma & Zhang 2015, Ma & al. 2016, Xu & al. 2017). During an ongoing mycological survey in these forests, an interesting hyphomycete with morphological features of *Cacumisporium* was collected on dead branches. It was found to be distinct from previously described taxa and is therefore proposed as new to science. The specimens are conserved in the Herbarium of the Department of Plant Pathology, Jiangxi Agricultural University, Nanchang, China (HJAUP).

Cacumisporium fusiforme Z.H. Xu, Jian Ma, X.G. Zhang & R.F. Castañeda, **sp. nov.** FIG. 1

MB 829269

Differs from *Cacumisporium sigmoideum* by its smaller, 3(–4)-euseptate, fusiform conidia with a rounded apex.

TYPE: China, Jiangxi Province, Jiulianshan National Nature Reserve, on dead branches of an unidentified broadleaf tree, 3 November 2014, J. Ma (**Holotype**, HJAUP M0307).

ETYMOLOGY: refers to the fusiform conidial shape.

COLONIES on the natural substratum effuse, hairy, brown to dark brown. Mycelium partly superficial, partly immersed, composed of branched, septate, smooth, subhyaline to pale brown hyphae. CONIDIOPHORES macronematous,

Fig. 1. *Cacumisporium fusiforme* (holotype, HJAUP M0307).
A–C. Conidiophores, conidiogenous cells, and conidia; D. Conidia.

mononematous, simple, erect, straight or flexuous, smooth, septate, cylindrical, often with 1–2 enteroblastic percurrent extensions, dark brown at the base, pale brown or subhyaline at the apex, 73–185 × 4–7 μm. Conidiogenous cells polyblastic, integrated, terminal, sympodial elongated, with flattened or inconspicuous denticles at the conidiogenous loci, brown, pale brown to subhyaline, 35–61.5 × 5.5–6.5 μm. Conidial secession schizolytic. Conidia solitary, acrogenous, fusiform, occasionally curved, 3(–4)-euseptate, end cells subhyaline or pale brown to pale yellow, middle cells yellow-brown, smooth, 23.5–33.5 × 7.5–9 μm, apex rounded, base truncate, 2–2.5 μm wide, accumulating in brown, somewhat mucilaginous masses.

Comments –Among *Cacumisporium* species, *C. fusiforme* is most similar to *C. sigmoideum* Mercado & R.F. Castañeda, which is distinguished by its falcate, mostly sigmoid, 3-euseptate, larger (26–41 × 7.6–10.2 μm) conidia with an acute apex (Mercado-Sierra & Castañeda-Ruíz 1987).

Key to *Cacumisporium* species

1. Conidia 1-euseptate, versicolorous, ovoid,
   18–26 × 11–16 µm ..... *C. uniseptatum*
1. Conidia 3(–4)- or 7-euseptate, botuliform, fusiform, obturbinate,
   sigmoid, obovoid to broadly ellipsoid 2
2. Conidia 7-euseptate,
   narrowly ellipsoid to somewhat fusiform, 26–32 × (3–)4(–5) µm ... *C. spooneri*
2. Conidia 3(–4)-euseptate 3
3. Conidia rugose to verrucose,
   obovoid to broadly ellipsoid, 28–35 × 14–20 µm ..... *C. rugosum*
3. Conidia smooth 4
4. Conidia botuliform, 16–22 × 4.5–5 µm ..... *C. capitulatum*
4. Conidia obturbinate, sigmoid or fusiform, at least 6 µm diam. 5
5. Conidia concolorous, broad fusiform, sometimes navicular or
   somewhat ellipsoid, 12–17 × 6–8 µm ..... *C. tropicale*
5. Conidia versicolor 6
6. Conidia with one swollen cell strongly pigmented,
   18–28 × 8–9 µm ..... *C. pleuroconidiophorum*
6. Conidia with two cells strongly pigmented 7
7. Conidia fusiform, 23.5–33.5 × 7.5–9 µm, 3(–4)-euseptate,
   with a rounded apex ..... *C. fusiforme*
7. Conidia falcate, mostly sigmoid, 26–41 × 7.6–10.2 µm, 3-euseptate,
   with an acute apex ..... *C. sigmoideum*

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Literature cited

Castañeda-Ruíz RF, Kendrick B. 1991. Ninety-nine conidial fungi from Cuba and three from Canada. University of Waterloo Biology Series 35. 132 p.

Castañeda-Ruíz RF, Heredia-Abarca G, Arias RM, Saikawa M, Minter DW, Stadler M. 2007a. Anamorphic fungi from submerged plant material: *Phaeomonilia pleiomorpha*, *P. corticola* and *Cacumisporium pleuroconidiophorum*. Mycotaxon 100: 327–336.

Castañeda-Ruíz RF, Gusmão LFP, Guarro J, Stchigel AM. 2007b. Two new anamorphic fungi from Brazil, *Cacumisporium tropicale* and *Acrodictys irregularis*. Mycotaxon 102: 91–99.

Hughes SJ. 1958. Revisiones hyphomycetum aliquot cum appendice de nominibus rejiciendis. Canadian Journal of Botany 36(6): 727–836. https://doi.org/10.1139/b58-067

Index Fungorum. 2018. Fungal names search [accessed 25 December 2018]. http://www.indexfungorum.org/names/Names.asp

Kirk PM. 1992. New or interesting microfungi XVI. Hyphomycetes from the British Isles. Mycotaxon 43: 231–236.

Ma J, Zhang XG. 2015. A preliminary report of dematiaceous hyphomycetes from dead branches in Jiangxi Province. Biological Disaster Science 38(4): 290–293.

Ma J, Xia JW, Zhang XG. 2016. Three new species of *Hemicorynespora* and *Solicorynespora* from southern China. Mycotaxon 131: 263–268. https://doi.org/10.5248/131.263

Mangenot F. 1953. Sur quelques hyphales dematiées lignicoles. Revue de Mycologie 18: 133–148.

Mercado-Sierra A, Castañeda-Ruíz RF. 1987. Nuevos o raros Hifomicetes de Cuba. I. Especies de *Cacumisporium*, *Gueda*, *Rhinocladium*, y *Veronaea*. Acta Botanica Cubana 50: 1–7.

Preuss CGT. 1851. Uebersicht untersuchter Pilze, besonders aus der Umgegend von Hoyerswerda. Linnaea 24: 99–153.

Rambelli A, Raimondo FM. 2014. Interesting dematiaceous hyphomycetes on *Lodoicea maldivica* dead fragments. Plant Biosystems 148(3): 467–472. https://doi.org/10.1080/11263504.2013.785449

Saccardo PA. 1877. Fungi italici autographice delineati. Michelia 1(1): 73–100.

Seifert K, Morgan-Jones G, Gams W, Kendrick B. 2011. The genera of hyphomycetes. CBS Biodiversity Series 9. 997 p.

Sutton B. 1973. Hyphomycetes from Manitoba and Saskatchewan, Canada. Mycological Paper 132. 143 p.

Tsui CKM, Goh TK, Hyde KD, Hodgkiss IJ. 2001. New species or records of *Cacumisporium*, *Helicosporium*, *Monotosporella* and *Bahusutrabeeja* on submerged wood in Hong Kong streams. Mycologia 93(2): 389–397. https://doi.org/10.2307/3761660

Wongsawas M, Wang HK, Hyde KD, Lin FC. 2009. Two new hyphomycetes from submerged wood collected in China. Sydowia 61(2): 345–351.

Xu ZH, Hu DM, Luo YQ, Ma J. 2017. Three species of *Linkosia* and *Spadicoides* new to China. Mycotaxon 132: 243–250. https://doi.org/10.5248/132.243

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Acarospora squamulosa, the correct name for *A. peliocypha*

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Abstract—The epithet of the species widely known as *Acarospora* "*peliscypha*" should be restored to its original orthography, *A. peliocypha*. The oldest name for this species is *Lichen squamulosus,* which has priority. *Lichen squamulosus* is lectotypified. *Acarospora squamulosa* replaces the later name *A. peliocypha*. *Acarospora squamulosa* is reported for continental North America from Baffin Island, Nunavut Territory, Canada.

Key words—*Acarospora rugulosa*, nomenclature, priority, taxonomy

Introduction

Although stability in nomenclature is desirable, it is not always achievable. Improvements in the understanding of taxonomy often necessitate changes in names, and the molecular revolution has caused, and will continue to cause, massive changes to the taxonomy of lichenized fungi, with corresponding changes in names. Another source of instability can be the need to achieve consensus about names. Consensus is achieved by applying the rules of the International Code of Nomenclature, as those rules are accepted throughout the botanical and mycological community, but "consensus" and "stability" come into conflict in cases where the rules imply that a commonly used name is not the correct one. In such cases, a judgment must be made about whether to accept a change in name or to invoke the cumbersome procedure of conservation to preserve the more familiar usage. In this paper we resurrect the name *Acarospora squamulosa* for the species currently known as *A. peliocypha*, which

frequently occurs in the literature as *A.* "*peliscypha*", a misspelling published by Theodore Fries (1860) based on the orthographic variant *Parmelia pelioscypha* listed by Wallroth (1831: 474). The orthographic variants *A. peliocypha* and *A. pelioscypha* are essentially synonyms: the first element from Greek pelios (πελιός) means "black; dark," and the alternative second elements are either from scyphos (σκύφος) meaning "cup" or from cyphos (κυφός) meaning "curved; the round of a cup." Since both variants are authentic, the original version *peliocypha* is not a correctable error and must be accepted.

The oldest epithet for this species is Schrader's *squamulosus*, which Acharius and other early 19th century authors accepted as having priority. It is unfortunate that the later epithet "*peliscypha*" was taken up following Fries's work. We do not consider that conservation would be appropriate in this case. We prefer to follow the Code of Nomenclature (which seems to us a matter of justice, too) and resurrect the older epithet.

Material & methods

Specimens from GZU, H, M and the private herbaria of Kocourková & Knudsen (hb. K&K) and Jiří Malíček (hb. Malíček) were studied using standard microscopy and spot tests. Specimens were sectioned by hand and measurements were made in water. Amyloid reactions of the hymenium were studied in fresh undiluted Merck Lugol's solution (IKI) following Knudsen & Kocourková (2018). The excellent description by A.H. Magnusson (1929) was revised. Macrophotographs were taken using an Olympus DP74 digital camera mounted on Olympus SZX 7 stereomicroscope equipped with PRO-SZM1-Focus Drive Motorization for stacking pictures and stacked using the Olympus DeepFocus 3.4 module. The figure plates were processed with QuickPhoto Camera 3.2 software fitted with Promicra Publisher Module and eventually refined with Adobe Photoshop CS4 Extended ver. 11.0.

Taxonomy

Acarospora squamulosa (Schrad.) Trevis., Rivista Period. Lav. Regia Accad. Sci. Lett. Arti Padova 1: 263. 1853. Fig. 1

≡ *Lichen squamulosus* Schrad., Ann. Bot. (Usteri) 22: 84. 1797.

Type: Systematische Sammlung Cryptogamischer Gewächse, Fascicle II, no. 153, Germany. Niedersachsen, Harz, on rocks (**lectotype designated here**, M! MBT385224; isolectotypes, GOTE! L!).

≡ *Parmelia squamulosa* (Schrad.) Ach., Methodus, sect. post.: 181. 1803.

= *Parmelia peliocypha* Wahlenb., in Acharius, Methodus, suppl.: 40. 1803.

Type: Norway. Lappland, Nord Cap, on rocks, Wahlenberg s.n. [type not seen or located].

≡ *Acarospora peliocypha* (Wahlenb.) Th. Fr. [as "*peliscypha*"], Lich. Arct.: 89. 1860.

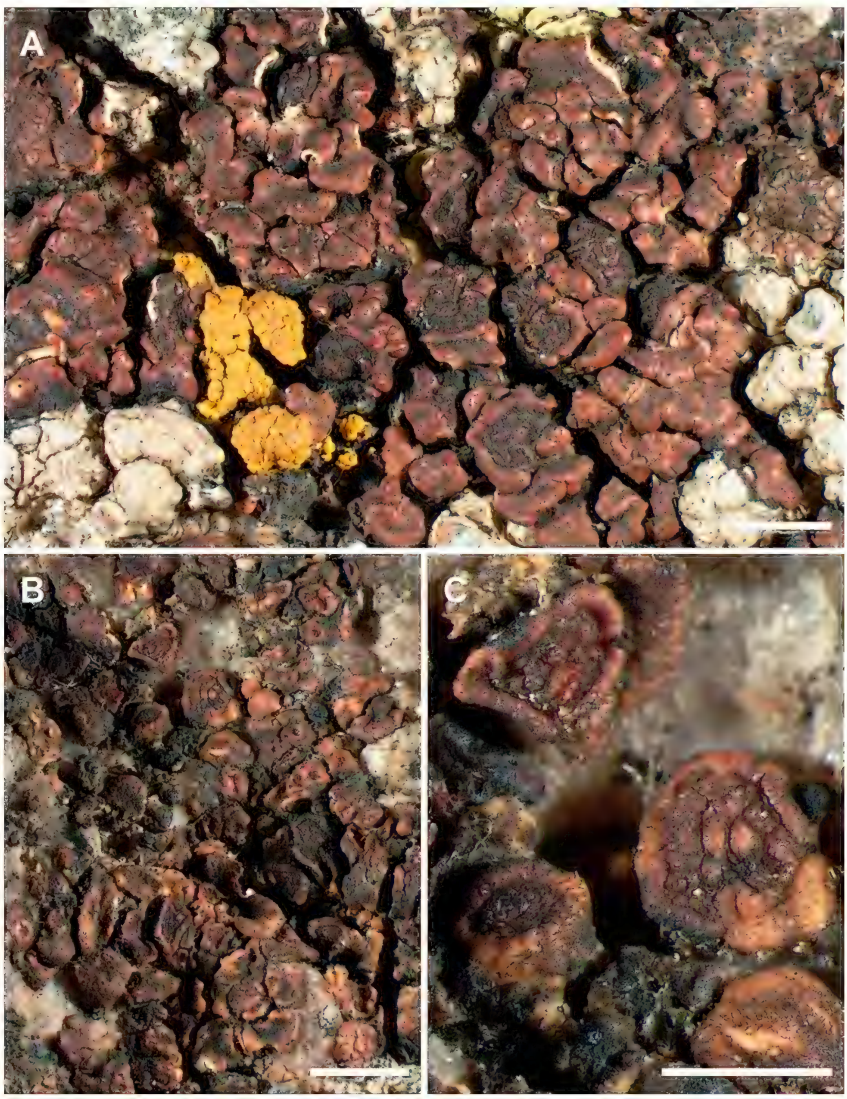

Fig. 1. *Acarospora squamulosa* (lectotype, Schrader No. 15, M): A. Subsquamulose imbricate thallus with apothecia; B. Crustose areolate thallus; C. Detail of apothecia with thalline margin and rugulose disc. Scale bars: A, B = 1 mm; C = 500 μm.

Thallus of areoles quite variable in size, as small as 0.5 mm and up to 3 mm diam., 100–500 μm thick, broadly attached, becoming sometimes lobulate and subsquamulose and sometimes imbricate, replicating by division. Upper

surface dark to light brown or reddish brown, shiny or matte, epruinose. Lower surface brown. Epicortex lacking or thin. Cortex 30–50 µm thick, upper layer brown, lower layer hyaline, cells round, 3–5 µm diam. Algal layer continuous to thin and scattered, 50–200 µm thick, algal cells 6–15 µm diam. Medulla thickening corresponding with areole size, hyphae thin-walled, 2–3 µm diam.

Apothecia usually 1 per areole, beginning immersed and (usually) becoming elevated in a thalline margin or expanding and thus reducing the areole to a thalline margin (pseudo-lecanorine), ≤1 mm diam., the disc epruinose, usually rugulose, usually round. Parathecium expanding around disc 10–30 µm without excluding thalline margin, 30–60 µm diam. Epihymenium 15–30 µm thick, coherent, brown to yellowish brown. Hymenium (including epihymenium but not accretions on disc surface) 100–120(–130) µm tall, hymenial gel IKI+ blue to red (hemiamyloid). Paraphyses 1.5–2 µm diam. at mid-level, apices unexpanded or expanded ≤3 µm. Asci 70–90 × 16–20 µm, 100–200 ascospores per ascus. Ascospores 3–6 × 1–2 µm. Subhymenium 25–50 µm thick, IKI+ blue (euamyloid). Hypothecium 10–30 µm tall. Pycnidia inconspicuous, ca. 100 × 60 µm, ampuliform conidiogenous cells 10–15 µm tall, conidia mostly 2 × 1 µm. Gyrophoric acid in cortex, C+, KC+ pinkish red in thin section, rarely cortical parts lacking a distinct reaction.

Selected specimens examined: **CANADA. Nunavut. Baffin Island**, Head of Clyde Fiord, on gneiss rock below a gyrfalcon nest, July 1950, M.E. Hale Jr. 372 (H). **CZECH REPUBLIC, Central Bohemia, Distr. Rakovnik**, Křivoklátsko Protected Landscape Area, Hracholusky, Čertova skála Nature Reserve, 49°59′52.535″N, 13°47′27.885″E, 345 m, on top of high outcrop above Berounka River, 14 July 1997, J. Kocourková 3727 (hb. K&K), Nezabudické skákly nature reserve, 50°1′10.02″N 13°51′2.34″E, 255 m, on spilite, 11 April 1996, J. Kocourková 9855 (hb. K&K), Roztoky, Na Babě Nature Reserve, 50°1′50.676″N, 13°52′18.069″E, 290 m, on steep rocky slope above Berounka River, on rhyolite, 17 June 1997, J. Kocourková 8471 (hb K&K), Týřovice, Týřov National Nature Reserve, Týřovické skály, w-facing rocks above Berounka River, 49°58′59″N 13°47′39″E, 330–420 m, on volcanic rock, 11 Jan. 2018, J. Malíček 12585 (hb Malíček). **Western Bohemia. Distr. Plzeň-sever**, Nečtiny, Preitenstein castle ruins, s- to sw-facing rocks below the castle, 49°57′37″N, 13°09′41″E., 550–580 m, on on spilite basalt, 13 April 2014, J. Malíček 7697 (hb Malíček). **FINLAND. Jämsä, Seppola**, 21 April 1934, A. Horkiverr (H); **Turku, Ruissalo Kalliolla**, on rock, 19 July 1922, V. Räsänen (H); **Kavidja**, on rock, 3 June 1908, K. Linkola (H). **NORWAY. Oppland, Ringebu**, near the village, on boulders in stone fence, 28 June 1925, A.H. Magnusson, Lichenes selecti scandinavici No 67 (H, GZU). **SWEDEN. Södermanland, Flemingsberg parish**, ca. 800 meters S of Masmo and just E of Lake Albysjön, 40 m, on S-facing exposed siliceous rock, 59°14′30″N 17°52′49″E, 10 April 2009, M. Westberg TU-3 (hb. K&K, 2 specimens). **RUSSIAN FEDERATION.** Kurkijoki [Kurkiyeki/Куркиеки], on schist, 8 August 1935, V. Räsänen (H). **Orenburg region, Mednogorsk district**, 14 km NE of Mednogorsk, vicinity of Yumaguzino the 2nd village, 51°32′41″N 58°38′50″E, 357 m, on basalt outcrops, 30 May 2013, A. Paukov (hb. K & K).

Ecology & distribution. Asia, Europe, North America, on various siliceous rock, on gneiss, rhyolite and basalt including spilite (Magnusson 1929, Golubkova 1988).

Discussion. Schrader's exsiccatae Systematische Sammlung Cryptogamischer Gewächse were published in two fascicles in 1796 and 1797. According to Sayre (1969), they did not include any descriptive text, although schedae for each fascicle were published in volumes 20 (1796) and 22 (1797) of Usteri's Annalen der Botanik. According to Stafleu & Cowan (1985), the schedae were also published in 1796 and 1797 as independent publications. It is not known therefore, whether the journal contains preprints or reprints, but we have assumed the former. We have not been able to consult the independent publications but assume that their text is word-for-word identical with the journal.

The name *Lichen squamulosus* was validly published in the journal adjacent to the number "153", an unambiguous reference to number 153 in the exsiccata. The name must therefore be typified on number 153 in the exsiccata. Because the exsiccata was issued in multiple copies, and we cannot be sure that each number represents a single gathering, a lectotype should be designated. We were able to locate only three specimens of number 153, one in the herbarium of Munich (M), one in Leiden (L), and one in Göttingen (GOET). The specimen in M is 7 cm in diameter and is designated here as the lectotype, as it can be easily studied without any damage. The specimen in L is too scanty for non-destructive microscopic and chemical examination, but does appear to belong to the same species, and is acceptable as an isolectotype. The specimen in GOET, which is smaller than the specimen in M and identified by F. Arnold who also did spot tests, is also an excellent isolectotype.

The lectotype and isolectotypes belong to the species widely known as either *Acarospora* "*peliscypha*" or *A. peliocypha*. Theodore Fries cited several names in synonymy, but as they are not validly published, proposed at an infra-specific rank, or cited with "p.p." or "?", his name is legitimate (Fries 1860). The correct spelling of Fries's epithet should be *peliocypha,* because he based his combination on *Parmelia peliocypha* Wahlenb., which he miscited as *P.* "*peliscypha.*" Acharius (1803) himself considered *Parmelia peliocypha* to be merely a synonym of *Parmelia squamulosa*; but Wahlenberg, who named *P. peliocypha*, regarded it as referring to a good species. ICN (Shenzhen) Art. 36.1, Ex 3 (Turland & al. 2018) supports the name as validly published. We could not locate Wahlenberg's collection at BM, H, UPS, or S or in the literature. Magnusson (1929) did not state that he actually saw Wahlenberg's specimens,

only that Wahlenberg eventually incorporated *P. peliocypha* "for the most part" into his concept of *Lichen cervinus* Wahlenb., which may explain the lack of annotated *peliocypha* type material. We choose not to neotypify the name *P. peliocypha,* because we believe a type may well be found among Wahlenberg specimens of *L. cervinus.* It is also possible the type actually represents the morphologically similar species currently known as *Acarospora rugulosa* Körb. We base our synonymy on the consensus of Acharius (1803), Arnold (1880), and Magnusson (1929) that Wahlenberg applied *P. peliocypha* to the species known as *L. squamulosus.*

As often happened in 19th century, names were often based on one-line descriptions without examining the types. In this case the name *Lichen squamulosus* was misapplied to the species *A. macrospora* A. Massal. ex Bagl. (Magnusson 1929). In his discussion of the nomenclature of *A. macrospora*, Magnusson stated that *L. squamulosus* in Schrader's type was "for the most part" the species later called *Parmelia peliocypha,* but he wrote "to not cause perpetual confusion it is best to let this name sink into oblivion" (Magnusson 1929). It seems fairer that we not propose to conserve the name *P. peliocypha* but recognize the nomenclatural priority of *Lichen squamulosus* and not let it sink into oblivion. We oppose conservation because E. Acharius, F. Arnold, and A.H. Magnusson all recognized *L. squamulosus* as the same species as *P. peliocypha* (Acharius 1803, Arnold 1880, Magnusson 1929). Magnusson arbitrarily decided to accept the name *P. peliocypha* even though he recognized that the taxon was originally called *L. squamulosus.*

Acarospora squamulosa is an arctic and montane species in Europe, although it has been collected at elevations as low as 255 m in the Czech Republic (Magnusson 1929). Collections have been identified by A.H. Magnusson from Fennoscandia to Russia in Europe (Magnusson 1929). Golubkova (1988) reported *A. squamulosa* in eastern Siberia in Asia, and Magnusson (1929) reported the species from Greenland in North America. We identified a specimen from Canada during this study but have seen no other specimens of *A. squamulosa* from continental North America. The taxon reported as *A.* "*peliscypha*" from California and the Sonoran region is rare and represents an undescribed species that needs further study (Knudsen 2007; for picture see Knudsen & al. 2017).

Acarospora squamulosa can be confused with *A. rugulosa* [= *A. montana* H. Magn.]. *Acarospora rugulosa* has a higher hymenium (110–)125–140(–175) µm vs. 100–120(–130) µm in *A. squamulosa,* and phylogenetic analysis supports *A. squamulosa* and *A. rugulosa* as distinct species (Westberg & al. 2015). The

two species can be identified accurately only by hymenium height, as they share overlapping morphological characters. *Acarospora rugulosa* is on the North American lichen checklist (Esslinger 2018) but we have yet to confirm this report.

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Literature cited

Acharius E. 1803. Methodus qua omnes detectos lichenes. Supplementum. Leipzig. 52 p.

Arnold F. 1880. Lichenologische Fragmente. XXII. Flora (Regensburg) 63: 371–385.

Esslinger TL. 2018. A cumulative checklist for the lichen-forming, lichenicolous and allied fungi of the continental United States and Canada. Version#22. Opuscula Philolichenum 17: 6–268.

Fries TM. 1860. Lichenes arctoi. Uppsala. 298 p.

Golubkova N. 1988. The lichen family *Acarosporaceae* Zahlbr. in the U.S.S.R. Leningrad: Nauka. (In Russian). 136 p.

Knudsen K. 2007. *Acarospora*. 1–38, in: TH Nash III & al. (eds). Lichen Flora of the Greater Sonoran Desert Region. Volume 3. Lichens Unlimited, Arizona State University, Tempe.

Knudsen K, Kocourková J. 2018. Two new calciphytes from Western North America, *Acarospora brucei* and *Acarospora erratica* (*Acarosporaceae*). Opuscula Philolichenum 17: 342–350.

Knudsen K, Lendemer JC, Schultz M, Kocourková J, Sheard JW, Pigniola A, Wheeler T. 2017. Lichen biodiversity and ecology in the San Bernardino and San Jacinto Mountains in southern California (U.S.A.). Opuscula Philolichenum 16: 15–138.

Magnusson AH. 1929. A monograph of the genus *Acarospora*. Kungliga Svenska Vetenskaps-Akademiens Handlingar 7(4). 400 p.

Sayre G. 1969. Cryptogamae exsiccatae: an annotated bibliography of published exsiccatae of *Algae*, *Lichenes*, *Hepaticae*, and *Musci*. Memoirs of the New York Botanical Garden 19. 174 p.

Stafleu FA, Cowan RS. 1985. Taxonomic literature, 2nd edition, vol. 5: Sal–Ste. The Hague & Boston. 1066 p.

Turland NJ, Wiersema JH, Barrie FR, Greuter W, Hawksworth DL, Herendeen PS, Knapp S, Kusber WH, Li DZ, Marhold K, May TW, McNeill J, Monro AM, Prado J, Price MJ, Smith GF (eds). 2018. International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017. Regnum Vegetabile 159. Glashütten: Koeltz Botanical Books. 254 p.

Wallroth FG. 1831. Flora cryptogamica germaniae, vol. 1. Filices, lichenastra, muscos et lichenes. Nuremberg. 654 p.

Westberg, M, Millanes AM, Knudsen K, Wedin M. 2015. Phylogeny of the *Acarosporaceae* (*Lecanoromycetes*, *Ascomycota*, *Fungi*) and the evolution of carbonized ascomata. Fungal Diversity 70: 145–158. https://doi.org/10.1007/s13225-015-0325-x

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Megalaria yunnanensis sp. nov. from Yunnan, China

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Abstract—*Megalaria yunnanensis* from southern China is described as a new species. The lichen is diagnosed by its bi-layered exciple, presence of atranorin, zeorin, and fumarprotocetraric acid in the thallus, ascospore size (20–25 × 5–7.5 µm), and the lack of indentations from the cell lumina into the center of the spore septum.

Key words —East Asia, *Lecanorales*, lichenized fungi, *Ramalinaceae*, taxonomy

Introduction

Megalaria (*Ramalinaceae*) was erected for the single species *M. grossa* (Pers. ex Nyl.) Hafellner (Hafellner 1984). The genus is recognized by its crustose thallus, biatorine apothecia, greenish to dark purple epihymenium, (2–)8-spored ascus, *Lecanora*-, *Bacidia*-, or *Biatora*-type asci, and 1-septate, ellipsoidal to subglobose, colourless, thick-walled ascospores (Ekman & Tønsberg 1996; Brodo & al. 2001: 428–429; Galloway 2004; Lendemer 2007; Sanderson 2009; Fryday & Lendemer 2010; McCarthy & Elix 2016; McMullin & Lendemer 2016).

Megalaria contains about 48 species worldwide (Lendemer & al. 2016, McMullin & Lendemer 2016, McCarthy & Elix 2016, Su & Ren 2017, Elix & McCarthy 2018). In China, only two species, *M. hainanensis* Q. Ren and *M. laureri* (Hepp ex Th. Fr.) Hafellner have been reported (Su & Ren 2017). During our study of the lichen flora of Mt. Wuliang in Jingdong county, Yunnan province, China, we collected a species of *Megalaria* new to science.

Materials & methods

The specimens were collected in Yunnan Province, China, and are preserved in Lichen Section of the Botanical Herbarium, Shandong Normal University, Jinan, China (SDNU). The specimens were examined morphologically using a COIC XTL7045B2 stereo-microscope and anatomically with an Olympus CX41 polarizing microscope and photographed under Olympus SZX16 and BX61 with DP72. Thallus and medulla were tested with K (a 10% aqueous solution of potassium hydroxide), C (a saturated solution of aqueous sodium hypochlorite) for identification. Lichen substances were identified using standardized thin layer chromatography techniques (TLC) with system C (Orange & al. 2001).

Total genomic DNA was extracted with DNeasy Plant Mini Kit according to the manufacturer's instructions and purified with PCR quick-spin™ PCR Product Purification Kit. The ITS1-5.8S-ITS2 regions were amplified in a C1000TM automatic thermocycler using primers ITS1F (Gardes & Bruns 1993) and ITS4 (White & al. 1990) in a 50 μL volume containing 5 units of 1 μl Taq DNA Polymerase (Sangon Biotech), 5 μL ITS buffer, 37.2μl ddH2O, 0.8 μL 10 μM F primer solution, 0.8 μL 10 μM R primer solution, 3.2 μL 2.5 mM per dNTP solution, and 2 μL genomic DNA. PCR thermal cycling parameters were initial denaturation at 98°C for 2 min, followed by 35 3-step cycles (98°C for 10 s, 54°C for 10 s, 72°C for 1 min), and a final 5 min extension at 72°C. PCR products were sanger-sequenced by Sangon Biotechnology Company.

All raw sequences were assembled and edited using SeqMan 7.0. The ITS data set comprised two newly generated sequences from the holotype and a paratype of our new species and eight relevant ITS sequences of *Megalaria* (plus two outgroup sequences) downloaded from GenBank. The sequences were aligned in MAFFT version 7 (Katoh & al. 2005) with the parameters set to default values. Ambiguous regions were excluded using Gblocks (Talavera & Castresana 2007) with default settings. There were 479 positions total in the final dataset. Maximum likelihood (ML) was used to reconstruct the phylogenetic tree (Fig. 2). *Biatora subduplex* (Nyl.) Printzen and *B. meiocarpa* (Nyl.) Arnold were used as outgroup (McMullin & Lendemer 2016). All characters were equally weighted and treated as unordered.

Taxonomy

Megalaria yunnanensis C.X. Wang & L. Hu, **sp. nov.** Fig. 1

MB 829259

Differs from *Megalaria albocincta* by its longer ascospores and its lack of indentations from the cell lumina into the center of the spore septum.

Type: China. Yunnan province, Jingdong county, Mt. Wuliang, 24°24′02″N 100°45′44″E, alt. 2200 m, on bark, 7 Aug. 2017, R. Tang 20170963 (**Holotype**, SDNU; GenBank MK348528).

Etymology: The specific epithet *yunnanensis* refers to Yunnan province, where this species was found.

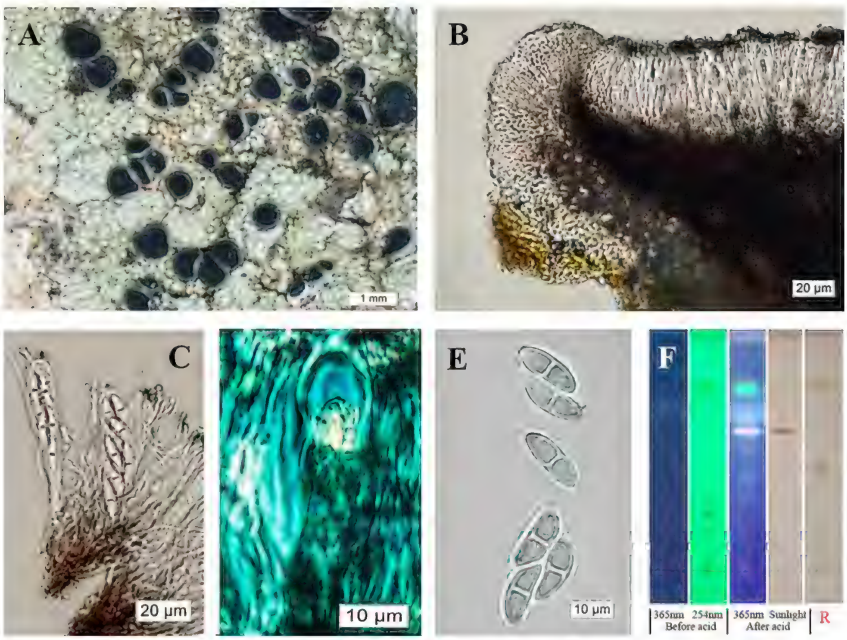

FIG. 1. *Megalaria yunnanensis.* (Holotype, SDNU (Tang 20170963)). A. Thallus and apothecia; B. Apothecial section; C. Asci and ascospores; D. Amyloid reaction of ascus, *Biatora*-type; E. Ascospores; F. TLC chromatograms (R = *Lethariella cladonioides*).

THALLUS crustose, verrucose protuberance, corticolous, white, without soredia. APOTHECIA biatorine, adnate, circular in outline, 0.38–1 mm diam., margins pale, waxy white, becoming excluded with age; DISCS black, flat, not pruinose, occasionally with few crystals. Epihymenium dark green to black, 22.5–52.5 μm tall, K–, N+ red; hymenium hyaline, 50–67.5 μm tall, K–, N–; subhymenium colourless to red-brown or black, 37.5–50 μm tall, K+ red, N+ red-brown to red; hypothecium black, 25–65 μm tall, K–, N+ red; paraphysis hyaline, simple, easily free, I+ blue. ASCI 8-spored, 45–62.5 × 11.25–15 μm, *Biatora*-type. ASCOSPORES ellipsoid, colourless, thick walled, 1-septate, not halonate, 20–25 × 5–7.5 μm (n = 32). PYCNIDIA not seen.

CHEMISTRY—Cortex and medulla K+ yellow, C–, KC–; thallus UV–; atranorin, zeorin, and fumarprotocetraric acid detected by TLC.

ADDITIONAL SPECIMENS EXAMINED: **CHINA. YUNNAN, Jingdong**, Mt. Wuliang, 24°24′02″N 100°45′44″E, alt. 2200 m, on bark, 7 Aug. 2017, R. Tang 20170805,

GenBank MK348527; 20170806; 20170820; 20170838; 20170856; 20170870; 20170896; 20170904; 20170918; 20170923; 20170926; 20170956; 20170962; 20170966 (SDNU).

Distribution—The type specimen was collected from Mt. Wuliang, Jingdong, Yunnan, which has a subtropical monsoon climate. The average annual temperature, relative humidity, and rainfall are 18.3°C, 77%, and 108.7 cm. This species grows on bark and is known only from the type locality.

Discussion

Megalaria yunnanensis is similar to *M. albocincta* (Degel.) Tønsberg, *M. alligatorensis* Lendemer, *M. anaglyptica* (Kremp.) Fryday & Lendemer, and *M. pulverea* (Borrer) Hafellner & E. Schreiner, all of which are characterized by a bi-layered exciple with a prosoplectenchymatous outer layer and an inner layer composed of a textura intricata with large intercellular spaces and a thalline chemistry of atranorin, zeorin, and fumarprotocetraric acid. However, the other four species are separated from *M. yunnanensis* by their shorter or smaller ascospores—*M. albocincta:* 13–17 × 6.5–8.5 µm, *M. alligatorensis:* 12–14 × 3.8–5.5 µm, *M. anaglyptica:* 17–22 × 4–6 µm, and *M. pulverea:* 10–16(–19) × 4.5–6.5 µm (Ekman & Tønsberg 1996, Kalb 2007, Sanderson 2009, Lendemer & al. 2016).

Furthermore, *M. albocincta* has two-sided indentations from the cell lumina into the center of the spore septum (Ekman & Tønsberg 1996); *M. alligatorensis* has a *Bacidia*-type ascus and greenish-blue thallus that forms small circular patches (Lendemer & al. 2016); *M. anaglyptica* has a thick granular thallus and dirty greyish brown subhymenium (Kalb 2007); and *M. pulverea* has a sorediate granular thallus and bigger apothecia (0.5–2.2 mm diam.; Sanderson 2009).

As shown in Fig. 2, *M. yunnanensis* is sister to *M. pulverea*, but the genetic distance (Kimura 2-parameter model) between these two species is quite large (0.198–0.207). Interestingly, a sterile sorediate species *M. alleniae* Lendemer & McMullin from southeastern North America (McMullin & Lendemer 2016) is chemically similar to *M. yunnanensis*, but our new species obviously differs from *M. alleniae* morphologically and molecularly (with a 0.212 genetic distance separating *M. alleniae* and *M. yunnanensis*).

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The authors thank Dr. J.C. Lendemer (Institute of Systematic Botany, New York Botanical Garden, Bronx, New York, USA) and Dr. Shou-Yu Guo (State Key Laboratory

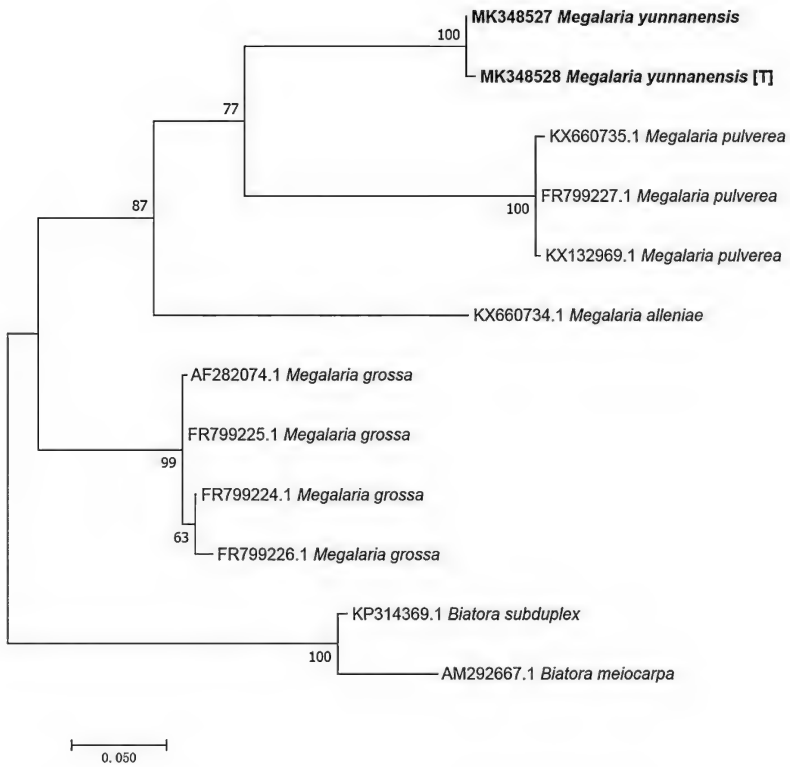

Fig. 2. Molecular phylogenetic tree illustrating the position of *Megalaria yunnanensis* in *Megalaria* s.l. based on ITS sequences. *Biatora* species were selected as the outgroup. Maximum likelihood bootstrap support values >50% are shown on nodes.

of Mycology, Institute of Microbiology, CAS, Beijing) for presubmission reviews and providing great help during the study. We also thank Dr. Alan M. Fryday (Michigan State University, USA) for providing suggestions and useful literature during the study. This work was supported by Emergency Management Project of National Natural Science Foundation of China (31750001), the National Natural Science Foundation of China (31400015), the National Natural Science Foundation of China Youth Science Foundation (31600100), and the Scientific Research Foundation of the Graduate School of Shandong Normal University (SCX201945) .

Literature cited

Brodo IM, Sharnoff SD, Sharnoff S. 2001. Lichens of North America. Yale University Press, New Haven & London. 795 p.

Ekman S, Tønsberg T. 1996. A new species of *Megalaria* from the North American West Coast, and notes on the generic circumscription. Bryologist 99: 34–42. https://doi.org/10.2307/3244435

Elix A, McCarthy M. 2018. Ten new lichen species (*Ascomycota*) from Australia. Australasian Lichenology 82: 20–59.

Fryday AM, Lendemer JC. 2010. Reassessment of the genus *Catillochroma* (lichenized *Ascomycota, Ramalinaceae*). Lichenologist 42: 587–600. https://doi.org/10.1017/S0024282910000320

Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for basidiomycetes—application to identification of mycorrhizae and rusts. Molecular Ecology 2: 113–118.

Galloway DJ. 2004. New lichen taxa and names in the New Zealand mycobiota. New Zealand Journal of Botany 42: 105–120. https://doi.org/10.1080/0028825X.2004.9512893

Hafellner J. 1984. Studien in Richtung einer natürlicheren Gliederung der Sammelfamilien *Lecanoraceae* und *Lecideaceae*. Beihefte zur Nova Hedwigia 79. 241–371.

Kalb K. 2007. New or otherwise interesting lichens. III. Bibliotheca Lichenologica 95: 297–316.

Katoh K, Kuma K, Toh H, Miyata T. 2005. MAFFT version 5: improvement in accuracy of multiple sequence alignment. Nucleic Acids Research 33: 511–518. https://doi.org/10.1093/nar/gki198

Lendemer JC. 2007. *Megalaria beechingii* (lichenized *Ascomycota*), a new species from eastern North America. Opuscula Philolichenum 4: 39–44.

Lendemer JC, Harris RC, Ruiz AM. 2016. A review of the lichens of the Dare Regional Biodiversity Hotspot in the Mid-Atlantic Coastal Plain of North Carolina, eastern North America. Castanea 81: 1–77. https://doi.org/10.2179/15-073R2

McCarthy M, Elix A. 2016. A new species of *Megalaria* (lichenized *Ascomycota*, *Ramalinaceae*) from north-eastern Queensland, Australia. Australasian Lichenology 79: 20–25.

McMullin RT, Lendemer JC. 2016. *Megalaria allenae* [sic] (*Ramalinaceae*), a new sorediate species from southeastern North America previously confused with *M. pulverea*. Bryologist 119(3): 290–297. https://doi.org/10.1639/0007-2745-119.3.290

Orange A, James PW, White FJ. 2001. Microchemical methods for the identification of lichens. British Lichen Society, London. 101 p.

Sanderson NA. 2009. *Megalaria*. 565–567, in: CW Smith & al. (eds). The Lichens of Great Britain and Ireland. British Lichen Society, London.

Su QX, Ren Q. 2017. A new species of *Megalaria* (*Ascomycota*, *Ramalinaceae*) and *M. laureri* new to mainland China Phytotaxa 313(1): 147–150. https://doi.org/10.11646/phytotaxa.313.1.13

Talavera G, Castresana J. 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Systematic Biology 56: 564–577. https://doi.org/10.1080/10635150701472164

White TJ, Bruns T, Lee S, Taylor JW. 1990. 38–Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. 315–322, in: MA Innis & al. (eds). PCR Protocols: a Guide to Methods and Applications. San Diego, CA: Academic Press. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

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The conserved type of *Lichen fuscatus* [≡ *Acarospora fuscata*]

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Abstract—The conserved type of *Lichen fuscatus,* the basionym of *Acarospora fuscata,* is described, and two genes, ITS and mrSSU, are made available through GenBank for further phylogenetic research.

Key words—*Acarospora gallica*, *A. variegata*, nomenclature, taxonomy

Introduction

Acarospora fuscata is the most common species of the genus in Europe (Magnusson 1929). In crustose lichen communities on siliceous rock, its pale brown color is distinctive, and it is often collected. But despite *A. fuscata* being common, it did not have a designated type or legitimate name (Arcadia & al. 2015). Because the taxon is common and the name *A. fuscata* is in wide use, a formal proposal was made to conserve the name *Lichen fuscatus* Schrad. (Arcadia & al. 2015) and has been accepted (May 2017). A modern conserved type that could be sequenced was designated that matched the species concept of A.H. Magnusson, who did the most extensive study of the species in Europe (Magnusson 1929). The taxon's most typical phenotype was collected in central Europe to serve as conserved type. In this paper, we present a description of the conserved holotype and isotypes and designate GenBank sequences to aid in the identification and phylogenetic study of *A. fuscata* and other *Acarospora* taxa containing gyrophoric acid that have been confused with it.

Material & methods

The description is based only on the conserved holotype and isotypes. Specimens were studied with standard microscopy. Measurements were made in water. Undiluted fresh Merck Lugol's (IKI) was used to test amyloid reactions of thin squashed sections of apothecia following Knudsen & Kocourková (2018). Ascus structure was studied with IKI with or without pretreatment with KOH (Hafellner 1993). Thin-layer chromatography (TLC) was used to verify the presence of secondary metabolites (Orange & al. 2001).

DNA was extracted from the holotype (Kocourková 8499, S) using the Invisorb Spin Plant Mini Kit. The fungal ITS rDNA and mitochondrial SSU were amplified with the following primers: ITS1F (Gardes & Bruns 1993) and ITS4 (White & al. 1990), and mrSSU1 and mrSSU3R (Zoller & al. 1999). PCR reactions of nrITS and mtSSU were prepared for a 20 µl final volume containing 14 µl double-distilled water, 4 µl MyTaq polymerase reaction buffer, 0.2 µl MyTaq DNA polymerase, 0.4 µl of each of the 25 mM primers, and 1 µl of the sample. Amplifications of both loci comprised an initial 1 min denaturation at 95 °C; 35 cycles of 1 min at 95 °C, 1 min at 56 °C, 1 min at 72 °C; and a final 7 min extension at 72 °C. The PCR products were visualized on a 0.8% agarose gel and cleaned with ExoSAP-IT, according to Thermo Fisher Scientific protocols. Both newly produced sequences, ITS (MK178225) and mrSSU (MK178225), were checked in BioEdit 7.2.5 (Hall 1999).

Macrophotographs were taken with an Olympus DP74 camera mounted on an Olympus SZX 7 stereomicroscope equipped with PRO-SZM1-Focus Drive Motorization for stacking pictures and stacked using the Olympus DeepFocus 3.4 module. The figure plates were processed with QuickPhoto Camera 3.2 software fitted with Promicra Publisher Module and eventually refined with Adobe Photoshop CS4 Extended ver. 11.0. The type locality picture was taken with Panasonic DMC LX5.

Taxonomy

Acarospora fuscata (Schrad.) Arnold,
Flora 53 (30–31): 469. 1871 ["1870"]. Fig. 1

≡ *Lichen fuscatus* Schrad., Spic. Fl. Germ.: 83. 16 Mai–5 Jun 1794, nom. cons. prop.

Type: Czech Republic. West Bohemia, Distr. Tachov, between Mezholezy and Racov, Přírodní park Sedmihoří (Natural Park), 49°37′53″N 12°51′41″E, 524 m, granite outcrops in *Pinus sylvestris* forest, 1 Oct. 2014, J. Kocourková 8499 & K. Knudsen (S, **conserved holotype**, designated in Arcadia & al. 2015; B, FH, KRAM, GZU, H, NY, PRA, UCR, UPS, conserved isotypes).

Thallus indeterminate of areoles, often contiguous, occasionally imbricate, covering areas sometimes more than a meter wide, or dispersed, sometimes forming lines along fractures in the rock. Areoles irregular in shape, often lobulate with edges free and often with a black margin, sometimes reduced to lecanorine-like apothecia, broadly attached to the substrate, replicating by division, 0.1–3.0 mm wide, up to 1 mm thick. Upper surface pale brown,

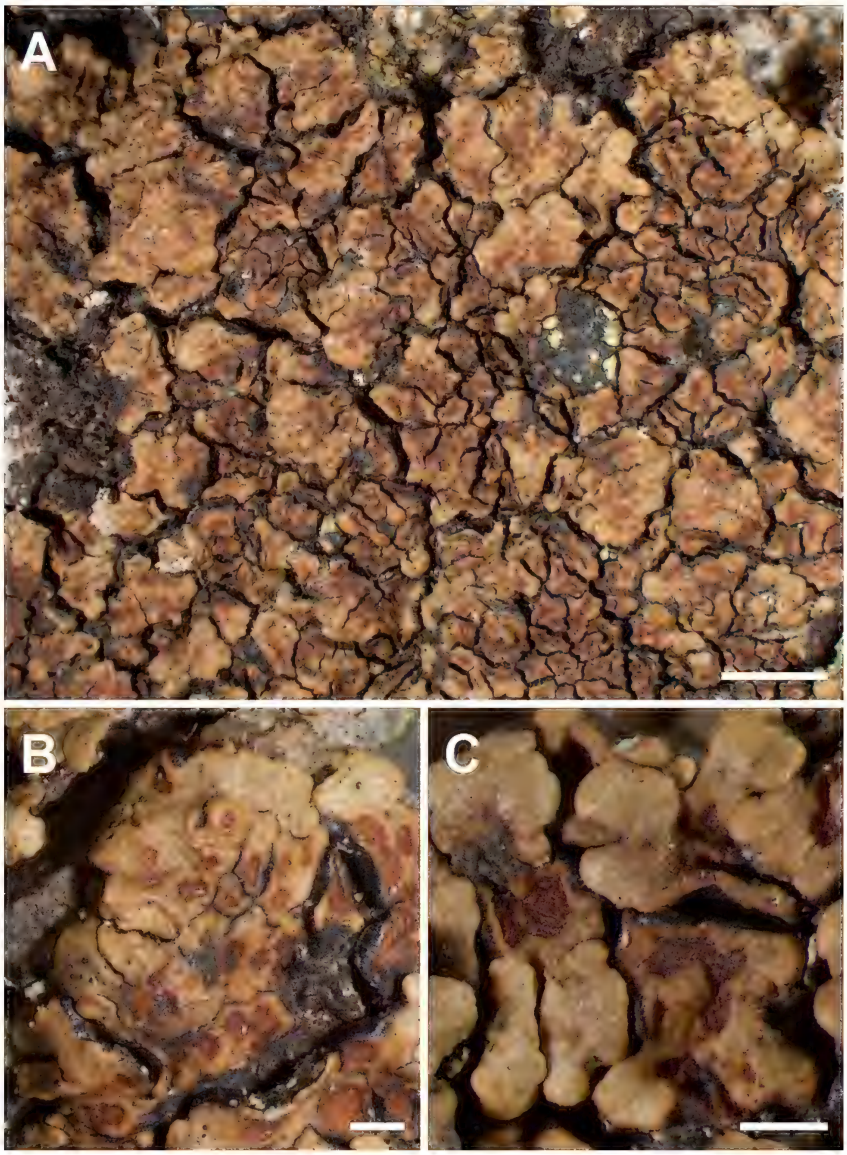

FIG. 1. *Lichen fuscatus* (conserved holotype, J. Kocourková 8499, S): A. Habit of the areolate thallus with apothecia; B. Marginal sublobate areole with sunken apothecia; C. Detail of areoles with apothecia. Scale bars: A = 2 mm; B, C = 500 µm.

epruinose, matte, smooth. Lower surface light or dark brown, corticate under the margins, becoming black through melanization. Epicortex thin (<10 µm

thick) or absent. Cortex 25–50 µm thick, upper layer yellow brown, lower layer hyaline, paraplectenchymatous, with cells 3–5 um diam., perpendicularly arranged and usually distinct, containing crystals visible in polarized light. Algal layer 50–100 µm thick, uninterrupted by hyphal bundles, with cells 5–12 µm diam. Medulla forming a thick mycelial base (gomphate), 0.1–0.8 mm thick, not distinctly stipitate, the outer surface usually carbonized, medullary hyphae intricate, thin-walled, septate, 3–4 µm thick.

Apothecia solitary or up to 10 per areole, immersed, punctiform 0.1–0.3 µm or expanding ≤0.6 mm wide, disc round or irregular, reddish-brown, darker than the thallus, epruinose, smooth or rough. Parathecium 10–20 µm diam., not expanding around the disc. Epihymenium 10–15 µm thick, reddish-yellow, coherent. Hymenium (80–)100–120(–140) µm high, paraphyses (1.0–)1.5–2.0 µm diam. at mid-level, not branching, septate, often with oil drops, with the apices barely expanded or capitate, 3–5 µm diam., hymenial gel IKI+ red or blue turning red (hemiamyloid). Asci 60–90 × 15–18 µm, ascus stain *Acarospora*-type, ascospores 4.0–5.0 × 1.5–2.0 µm, narrowly ellipsoid to ellipsoid. Subhymenium 30–60 µm thick, IKI+ blue (euamyloid). Hypothecium 10–20 µm thick. Pycnidia 100–110 × 60–70 µm, conidiogenous cells ampulliform, conidia 1.0–2.0 × 0.5–1.0 µm. Secondary metabolite: gyrophoric acid in cortex, C+, KC+ pinkish red in thin section. Sequences available in GenBank: ITS (MK178225) and mrSSU (MK178225).

Ecology & Substrate—The conserved type was collected at a low (310 m) elevation in full sunlight in *Pinus sylvestris* forest, where it covered the tops of granite outcrops without other associated lichens (Fig. 2)

Comments—Using our description and simple spot tests many specimens of *A. fuscata* can now be easily identified. Our description based on the conserved type does not differ significantly from the descriptions of Magnusson (1929) or Fletcher & al. (2009), except with respect to hymenium height. In the type the hymenium measures (80–)100–120(–140) µm, varying 60 µm in height depending on apothecial size. Magnusson (1929) gives a height of (70–)85–100 µm, a range of 30 µm. The British flora cites (70–)80–120 µm, a range of 50 µm (Fletcher & al. 2009).

Nonetheless, *Acarospora fuscata* exhibits a wide phenotypic variability in Europe, which led to the description of nine forms and one variety (Magnusson 1929). Now that we have gene sequences available from the conserved type of *A. fuscata,* unusual specimens can be verified with molecular analyses. Additional sequences can also be made from the conserved type or matching specimens, so that researchers can finally

FIG. 2. *Acarospora fuscata* type locality in West Bohemia, Czech Republic

establish if *A. fuscata* is polymorphic or a complex of several semi-cryptic or cryptic taxa. Candidates for a full genomic study can be selected that might reveal whether there is horizontal gene transfer with other *Acarospora* species. In Europe, the current taxonomic circumscriptions of *A. gallica* H. Magn. and *A. variegata* H. Magn. can also be revised. The British submontane taxon "*A. peliscypha*" sensu auct. Brit., included in *A. fuscata,* can be analyzed as well as pruinose specimens (Fletcher & al. 2009). The name *A. fuscata*, like other old European names, was applied to brown C+ red *Acarospora* taxa around the world. Additionally, collections from North America, Australia, or Asia identified as *A. fuscata* can be revised (Knudsen 2007, McCarthy & Elix 2017, Ohmura & Kashiwadani 2018).

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Literature cited

Arcadia L, Knudsen K, Westberg M. 2015. (2341) Proposal to conserve the name *Lichen fuscatus* Schrad. (*Acarospora fuscata*) against *L. fuscatus* Lam. with a conserved type (lichenised *Ascomycota*: *Acarosporaceae*). Taxon 64: 168–169. https://doi.org/10.12705/641.7

Fletcher A, James PW, Purvis, OW. 2009. *Acarospora* A. Massal. (1852). 125–132, in: CW Smith & al. (eds.) The lichens of Great Britain and Ireland. London: British Lichen Society.

Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for basidiomycetes. Application to the identification of mycorrhizae and rusts. Molecular Ecology 2: 113–118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x

Hafellner J. 1993. *Acarospora* und *Pleopsidium* – zwei lichenisierte Ascomycetengattungen (*Lecanorales*) mit zahlreichen Konvergenzen. Nova Hedwigia 56: 281–305.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.

Knudsen K. 2007 ["2008"]. *Acarospora*. 1–38, in: TH Nash III & al. (eds). Lichen Flora of the Greater Sonoran Desert Region. Volume 3. Lichens Unlimited, Arizona State University, Tempe, Arizona.

Knudsen K, Kocourková J. 2018. Two new calciphytes from western North America, *Acarospora brucei* and *Acarospora erratica* (*Acarosporaceae*). Opuscula Philolichenum 17: 342–350.

Magnusson AH. 1929. A monograph of the genus *Acarospora*. Kungliga Svenska Vetenskaps-Akademiens Handlingar, ser. 3, 7. 400 p.

May TW. 2017. Report of the Nomenclature Committee for Fungi: 20. Taxon 66: 483–495. https://doi.org/10.12705/662.15

McCarthy P, Elix JA. 2017. Two new species and a new record of *Acarosporaceae* (lichenized *Ascomycota*) from eastern Australia. Australasian Lichenology 80: 16–27.

Ohmura Y, Kashiwadani H. 2018. Checklist of lichens and allied fungi of Japan. National Museum of Nature and Science Monographs 49. 140 p.

Orange A, James PW, White FJ. 2001. Microchemical methods for the identification of lichens. London: British Lichen Society. 101 p.

White TJ, Bruns T, Lee S, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. 315–322, in: PCR Protocols: a Guide to Methods and Applications. MA Innes & al. (eds): Academic Press, New York. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Zoller S, Scheidegger C, Sperisen C. 1999. PCR primers for the amplifications of mitochondrial small subunit ribosomal DNA of lichen-forming *Ascomycetes*. Lichenologist 31: 511–516. https://doi.org/10.1006/lich.1999.0220

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Beltraniopsis cyclobalanopsidis sp. nov. from Guizhou, China

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Abstract—A new species, *Beltraniopsis cyclobalanopsidis*, is described and illustrated from a specimen collected on dead twigs of *Cyclobalanopsis stewardiana* [≡ *Quercus stewardiana*] in Guizhou Province, China. The fungus is characterized primarily by its longer, setiform conidiophores with inflated apexes. A dichotomous key to *Beltraniopsis* species is provided.

Key words—anamorphic fungi, lignicolous fungi, taxonomy

Introduction

Batista & Bezerra (1960) erected *Beltraniopsis* based on the type species, *Beltraniopsis esenbeckiae* Bat. & J.L. Bezerra. The genus is characterized by macronematous, simple or branched setiform conidiophores and polyblastic, discrete, sympodial extended, denticulate conidiogenous cells with separating cells that produce solitary, acropleurogenous, aseptate, biconic, smooth, brown conidia.

Beltraniopsis currently contains ten valid species found as saprobes on rotten leaves (Matsushima 1971, 1993; Pirozynski 1972; Castañeda-Ruiz & Arnold 1985; Rambelli & Ciccarone 1985; Gusmão & al. 2000; Castañeda Ruiz & al. 2006; Crous & al. 2014; Lin & al. 2017). A survey of saprobic microfungi on

dead twigs from a subtropical forest in Guizhou Province, China, has revealed a previously undescribed *Beltraniopsis* species. The specimen is deposited in the Mycological Herbarium of Zunyi Normal College, Zunyi, China (HMZNC).

Beltraniopsis cyclobalanopsidis Z.J. Xiao & Xiao X. Li, **sp. nov.** FIG. 1

FN570598

Differs from all other *Beltraniopsis* species by its longer setiform conidiophores inflated rather abruptly at the apical cell.

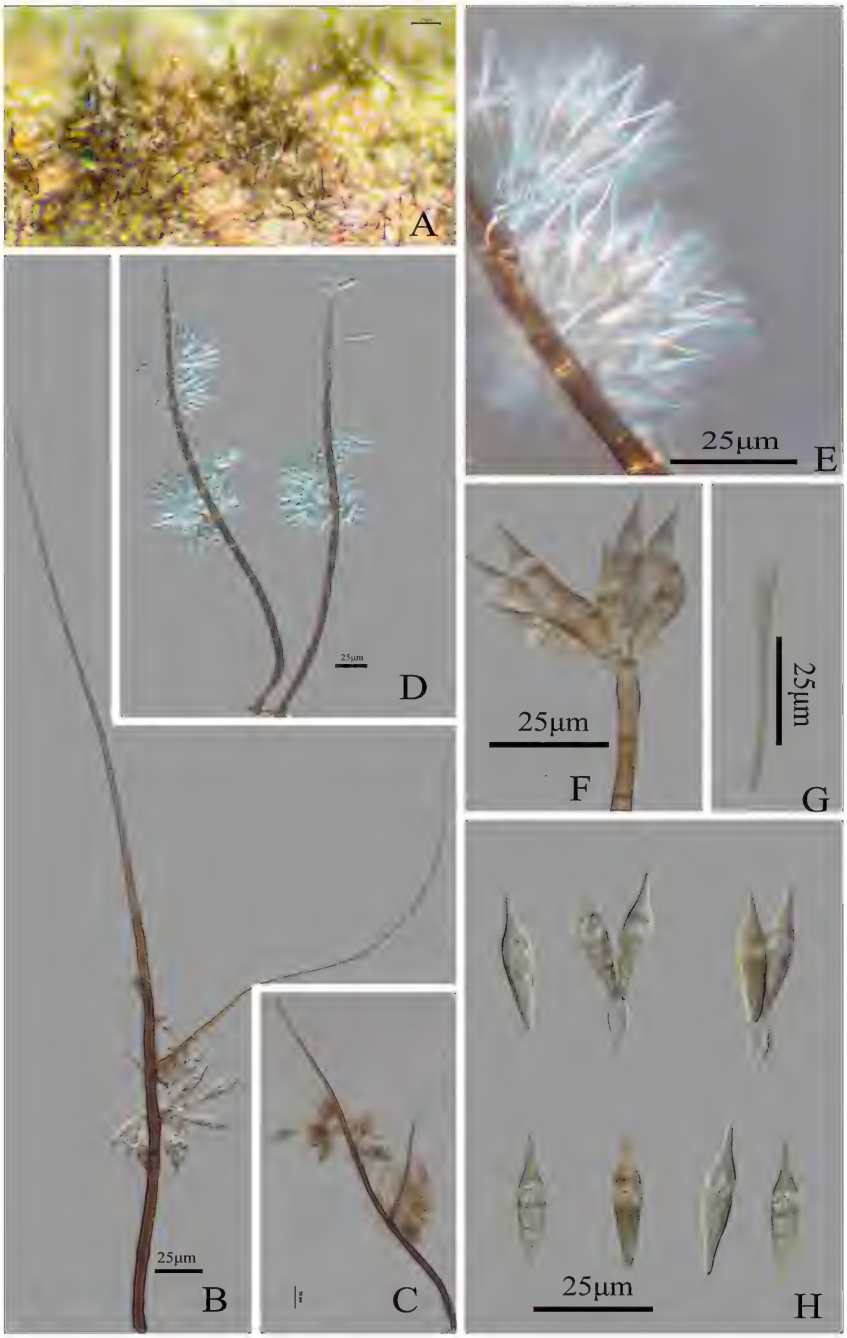
TYPE: China, Guizhou Province: Xishui National Nature Reserve, saprobic on dead twigs of *Cyclobalanopsis stewardiana* (A. Camus) Y.C. Hsu & H.W. Jen [≡ *Quercus stewardiana* A. Camus], 6 Jun. 2018, X.X. Li (**Holotype**, HMZNC 0450).

ETYMOLOGY: Latin, referring to the substrate genus *Cyclobalanopsis*.

COLONIES on dead twigs effuse, velutinous, brown to dark brown. Mycelium partly superficial and immersed in the substrate, composed of branched, septate, pale brown to brown, cylindrical, smooth-walled hyphae. CONIDIOPHORES macronematous, mononematous, setiform, sometimes becoming fertile at the apex, erect, slightly flexuous, unbranched or mostly branched in the lower-middle part, thick-walled, cylindrical, 13–25-septate, smooth or sometimes the lower-middle part with verrucose, arising from flat, radially lobed basal cells, brown to dark brown at the lower part, pale brown to hyaline at the upper part, 420–1110 µm long, 5.5–7.5 µm diam. at the base, tapering to the upper part, but apical cell inflated rather abruptly, rounded, thin-walled, hyaline, 2.2–4.5 µm diam. CONIDIOGENOUS CELLS polyblastic, mostly discrete intercalary, sometimes terminal, integrated, located in the lower-middle part of conidiophores, sympodial extended, 1–3-denticulate apex, smooth, ampulliform, flask-shaped, pale brown, 9.5–12.5 µm long, 5.5–7 µm diam. in the broadest part. SEPARATING CELLS ellipsoidal to ovoid, thin-walled, smooth, hyaline, denticulate apex, 7–10.5 µm long, 4.5–5.5 µm diam. in the broadest part. CONIDIA solitary, biconic to broad navicular, smooth, light brown, constricted at the supra-equatorial zone with subhyaline transverse band, 29–35 × 5.5–7.5 µm, apex rostrate, 0.5–1.5 µm wide, base obtuse.

COMMENTS—Based on the synopsis and key to *Beltraniopsis* species, *B. cyclobalanopsidis* is superficially similar to *B. longiconidiophora* C.G. Lin & K.D. Hyde and *B. miconiae* Gusmão & Grandi. *Beltraniopsis longiconidiophora*

FIG. 1. *Beltraniopsis cyclobalanopsidis* (holotype, HMZNC 0450). A. Conidiophores arising from twig surface; B, C. Conidiophores, conidiogenous cells, separating cells, and conidia; D. Conidiophore with broken upper part; E. Conidiogenous cells, separating cells, and conidia; F. Conidia arising from conidiogenous cells; G. Inflated apex of the conidiophores; H. Conidia.


A
25µm
E
25µm
D
25µm
F
25µm
G
25µm
B
C
25µm
H

differs from *B. cyclobalanopsidis* by its shorter setiform conidiophores (100–680 µm) tapering to a pointed apex and by its shorter conidia (18–29 µm; Lin & al. 2017); and *B. miconiae* differs by its much shorter setiform conidiophores (77–147 µm) with a slightly inflated apical cell (2–3.5 µm) and by its smaller separating cells (6–8 × 3–4.6 µm; Gusmão & al. 2000).

Key to *Beltraniopsis* species

1. Setiform conidiophore acute at the apex 2
1. Setiform conidiophore rounded or inflated at the apex 6
2. Setiform conidiophore verrucose ..... *B. asperisetifera*
2. Setiform conidiophore smooth 3
3. Setiform branched conidiophore apex not fertile 4
3. Setiform unbranched conidiophore apex fertile 5
4. Conidia 3–8.5 µm diam. ..... *B. longiconidiophora*
4. Conidia 4–5 µm diam. ..... *B. ramosa*
5. Conidia 20–25 × 4–6 µm ..... *B. esenbeckiae*
5. Conidia 22–28 × 6.5–8.5 µm ..... *B. tanzaniensis*
6. Setiform conidiophore rounded at the apex 7
6. Setiform conidiophore inflated at the apex 9
7. Conidia without rostrum and transverse band ..... *B. aquatica*
7. Conidia with rostrum and transverse band 8
8. Conidia 30–37 × 9 µm, separating cells 4–4.5 × 4 µm ..... *B. fabularis*
8. Conidia 30–33 × 7–8 µm, separating cells 7–10 × 4–6 µm ..... *B. neolitseae*
9. Conidia without rostrum and transverse band ..... *B. rhombispora*
9. Conidia with rostrum and transverse band 10
10. Setiform conidiophore 77–147 µm long ..... *B. miconiae*
10. Setiform conidiophore 420–1110 µm long ..... *B. cyclobalanopsidis*

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Literature cited

Batista AC, Bezerra JL. 1960. *Beltraniopsis* – novo gênero de fungos dematiaceae. Publicações do Instituto de Micologia da Universidade do Recife 296: 1–13.

Castañeda RF, Arnold GRW. 1985. Deuteromycotina de Cuba. I. Hyphomycetes. Revista del Jardín Botánico Nacional Universidad de la Habana 6: 47–67.

Castañeda Ruiz RF, Minter DW, Stadler M, Saikawa M, Camino-Vilaró M. 2006. Anamorphic fungi from submerged leaves in Cuba: *Brevicatenospora enteroproliferata* gen. et sp. nov. and *Beltraniopsis aquatica* sp. nov. Mycotaxon 96: 151–158.

Crous PW, Shivas RG, Quaedvlieg W, et al. 2014. Fungal Planet description sheets: 214–280. Persoonia 32: 184–306. https://doi.org/10.3767/003158514X682395

Gusmão LFP, Grandi RAP, Milanez AI. 2000. A new species of *Beltraniopsis* from Brazil, with a key to the known species. Mycological Research 104: 251–253. https://doi.org/10.1017/S0953756299001239

Lin CG, Hyde KD, Lumyong S, McKenzie EHC. 2017. *Beltrania*-like taxa from Thailand. Cryptogamie, Mycologie 38: 301–319. https://doi.org/10.7872/crym/v38.iss3.2017.301

Matsushima T. 1971. Microfungi of the Solomon Islands and Papua-New Guinea. Published by the author: Kobe, Japan.

Matsushima T. 1993. Matsushima mycological memoirs no. 7. Published by the author: Kobe, Japan.

Pirozynski KA. 1972. Microfungi of Tanzania. I. Miscellaneous fungi on oil palm. II. New hyphomycetes. Mycological Papers 129. 64 p.

Rambelli A, Ciccarone C. 1985. Two new dematiaceous hyphomycetes from humid tropic forest litter. Giornale Botanico Italiano 119: 291–294. https://doi.org/10.1080/11263508509426684

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Perichaena acetabulifera sp. nov. from Juarez City, Mexico

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Abstract—*Perichaena acetabulifera* is described as a new species of myxomycete that fruited profusely in moist chamber cultures on bark samples collected from *Populus deltoides* and *Ulmus pumila* growing in urban areas of Juarez City, Chihuahua, Mexico. Photographs of its diagnostic macroscopic and microscopic features are provided.

Key words—*Amoebozoa*, myxobiota, SEM, taxonomy, *Trichiales*, *Trichiaceae*

Introduction

The city of Juarez is located in the northern part of the Mexican state of Chihuahua, adjacent to El Paso (Texas) and Las Cruces (New Mexico), both in the United States. The city has an extreme climate, with temperatures ranging from 25 to 40°C in the summer and –4 to 13°C in the winter. In the spring, it is frequently lashed by strong winds that reach speeds up to 100 km/h. The dominant vegetation surrounding the city consists of xerophytic shrubs, with species of *Larrea* (*Zygophyllaceae*), *Yucca* (*Agavaceae*), *Opuntia* (*Cactaceae*), and *Prosopis* (*Mimosaceae*) as the prominent taxa, while the city interior contains a large number of ornamental plants in several recreational parks and gardens. These include species of *Cupressus*, *Thuja*, *Pinus*, *Morus*, *Populus*, *Fraxinus*, *Salix*, *Melia*, *Nerium*, *Vitex*, *Platanus*, *Tamarix*, *Chilopsis*, and *Quercus*. Several studies are being carried out to determine which myxomycetes develop on the bark of these urban plants, and the first results were published by Lizárraga & al. (2015, 2018).

Perichaena Fr. is characterized by its fructifications: sessile, stipitate or short plasmodiocarps. Generic characters include a simple peridium with a membranous inner surface and a granular and fragile outer surface that sometimes is crusted with calcium oxalate. The spores are yellow or reddish brown. The capillitium, which comprises tubular filaments with irregular or regular constrictions, is superficially ornamented with warts or spines and lacks spirals and birefringence (Nannenga-Bremekamp 1991). Although the absence of spiral-like bands in the capillitium is the most distinctive character separating *Perichaena* from *Trichia* Haller and *Oligonema* Rostaf., some species lacking a capillitium are placed within *Perichaena*, including *P. heterospinispora* Novozh. & al. (Novozhilov & al. 2008), *P. taimyriensis* Novozh. & Schnittler (Novozhilov & Schnittler 2001), *P, pachyderma* D.W. Mitch. & al. (Mitchell & al. 2011), and *Perichaena nigra* D. Wrigley & al. (Lado & al. 2014).

According to Lado (2018), 34 species are currently recognized worldwide, of which 13 have been recorded for Mexico (Moreno & al. 2007, Estrada-Torres & al. 2009, Rojas & al. 2010, Mitchell & al. 2011, Lizárraga & al. 2016).

Materials & methods

Samples for light microscopy were mounted in Hoyer's medium. Macroscopic photographs were taken with a Nikon SM2800 microscope, micrographs were obtained with a Nikon Eclipse 2000 microscope, and SEM micrographs were taken with a Zeiss DSM–950 microscope after critical point drying and sputtering, following Moreno & al. (2015). The specimens are stored in the Herbarium, Universidad de Alcalá, Madrid, Spain (AH) and the Herbarium, Instituto de Ciencias Biomédicas, Universidad Autónoma de Ciudad Juárez, Chihuahua, México (UACJ). Nomenclature follows Lado (2018).

Taxonomy

Perichaena acetabulifera Lizárraga, G. Moreno & Flores-Rom., **sp. nov.** PLATE 1

MB 829449

Differs from *Perichaena vermicularis* by its peridial inner surface and capillitium producing ocelli and craters.

TYPE. Mexico: Chihuahua, Juarez City, Biomedic Science Institute (Spanish acronym: ICB), on bark of *Ulmus pumila* L., placed into moist chamber 19–VIII–2015, obtained 22–IX–2015, leg. I. Flores-Romero & M. Lizárraga (**Holotype**, AH 49096; **isotype**, UACJ 2789).

ETYMOLOGY: *acetabulifera* from the Latin *acetabulum*, referring to a shallow concave vessel used as a cruet (and therefore the name given by Plinio to the suckers of an octopus) and *fer/fero* meaning to carry. The name draws attention to the resemblance of the capillitium and internally ornamented peridium to the suckers of octopus tentacles.

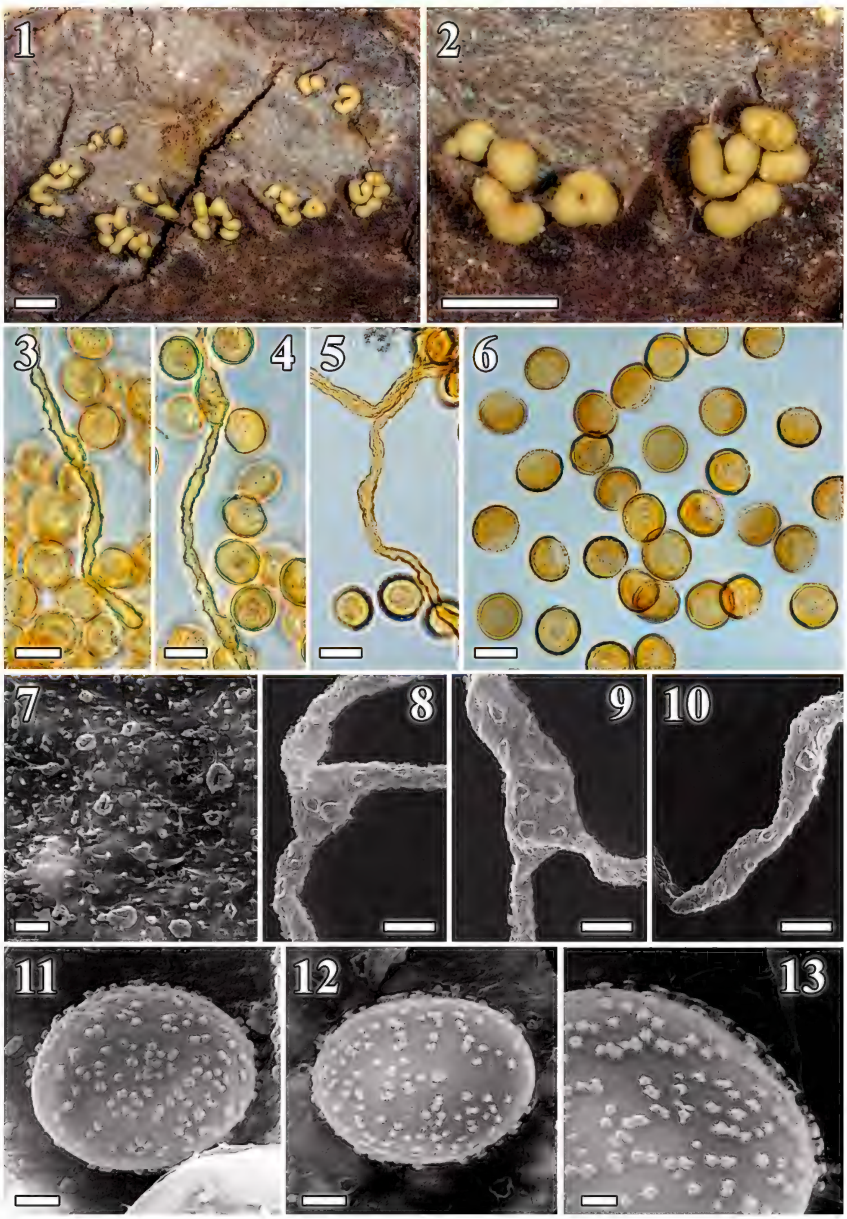

Plate 1 *Perichaena acetabulifera* (holotype, AH 49096): 1. Fructifications; 2. Detail of fructifications; 3–5. Capillitium by LM; 6. Spores; 7. Detail of the inner surfaces of peridium by SEM; 8–10. Detail of capillitium with craters by SEM; 11–12. Spores by SEM; 13. Detail of spore ornamentation by SEM. Scale bars: 1, 2 = 1 mm; 3–6 = 10 μm; 7–12 = 2 μm; 13 = 1 μm.

Fructifications scattered to gregarious, sessile, occasionally substipitate, globose, subglobose to short plasmodiocarpic, in this case somewhat sinuous, occasionally centrally depressed, to 0.8–1.5 × 0.2–0.6 mm. Stipe absent, occasionally with a blackish-brown small base up to 0.1 mm total height. Peridium simple, membranous, olivaceous yellow, inner part with papillae and small crests and craters that project over the surface. The outer surface granulose with deposits of amorphous material, brownish to olivaceous brown. Hypothallus absent or barely noticeable. Capillitium 2–6 µm in diam., somewhat abundant in the sample studied, formed by filaments with irregular edges, branched, with ocellus-like structures and craters, giving it a pitted appearance under OM. By SEM a capillitium lacking spines and reticulum can be observed, with rare and minute warts, and isolated small ocelli and craters of variable size, <2.5 µm in diam., resembling the suckers of an octopus. Spores 11–14(–16) µm in diam., ochraceous in mass, yellowish ochre by OM, globose, subglobose to oval, with a paler area, with sparse and irregular warts. By SEM the spore ornamentation is formed by warts with a flattened apex or bacula, unevenly distributed over the surface.

Other material examined: **MEXICO. Chihuahua, Juarez City**: Federal Public Park El Chamizal, on the bark of *Ulmus pumila*, placed into moist chamber 18–VIII–2015, obtained 29–IX–2015, leg. I. Flores-Romero & M. Lizárraga (UACJ 2827); on bark of *Populus deltoides* Marshall, placed into moist chamber 16–VIII–2016, obtained 30–VIII–2016 (UACJ 3317); obtained 20–IX–2016 (UACJ 3318, AH 49095); obtained 27–IX–2016 (UACJ 3319); obtained 17–X–2016 (UACJ 3320, AH 49100; UACJ 3321) obtained 18–X–2016 (UACJ 3322); obtained 31–X–2016 (UACJ 3323); obtained 10–XI–2016 (UACJ 3324); obtained 11–XI–2016 (UACJ 3325); obtained 10–XII–2016 (UACJ 3326). Institutes of Engineering, Technology, Architecture, Art and Design (IIT-IADA), on bark of *Ulmus pumila*, placed into moist chamber 19–VIII–2016, obtained 14–X–2016, leg. I. Flores-Romero & M. Lizárraga (UACJ 3327; UACJ 3328). Institute of Biomedical Sciences, on bark of *Ulmus pumila*, placed into moist chamber 19–VIII–2015, obtained 14–IX–2015, leg. I. Flores-Romero & M. Lizárraga (UACJ 2717); obtained 21–IX–2015 (UACJ 2773); obtained 22–IX–2015 (UACJ 2790, AH 49097); *Populus deltoides*, obtained 22–XI–2016, leg. I. Flores-Romero & M. Lizárraga (UACJ 3329, AH 49099); obtained 6–IX–2017 (UACJ 3307); obtained 13–IX–2017 (UACJ 3330); placed into moist chamber 9–IV–2018, obtaining fructifications 23–IV–2018 (UACJ 3063, AH 49098).

Observations—*Perichaena acetabulifera* is characterized by its sporocarpic to plasmodiocarpic fructifications and especially by its papillate peridium with isolated ocelli and craters, and a capillitium also with ocelli and craters, with rare warts visible only by SEM. The morphologically closest species are described below.

Perichaena vermicularis (Schwein.) Rostaf. is similar to the new species in its plasmodiocarpic fructifications, spore measurements, and the presence of papillae on the inner peridial and capillitial surfaces (Rammeloo 1981), but it lacks the ocelli and craters found in the new species.

Perichaena areolata Rammeloo is a foliicolous species with short sporothecae and craters and ocelli in the capillitium, similar to the new species. However, it can be separated its spherical areolated sporothecae, inner peridial surface lacking craters and ocelli (Rammeloo 1981), and its capillitium ornamented by spines or small appendixes.

Perichaena poronema Yu Li & H.Z. Li is distinguished by its globose sporothecae that are sometimes pulvinate, its circumscissile dehiscence by a definite preformed lid, its minutely warted and incompletely reticulated capillitium that is occasionally covered with holes, and small (8.75–9.5 µm in diam.) spores (Li & al. 1990).

Perichaena stipitata Lado & al. is a succulenticolous species that shares with *P. acetabulifera* the presence of craters or ocelli on the inner peridium, but which can be distinguished by its stalked fructifications and an inner peridium lacking papillae and crests but with dense ocelli and craters. Moreover, the capillitium has holes but lacks craters and ocelli.

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Literature cited

Estrada-Torres A, Wrigley de Basanta D, Conde E, Lado C. 2009. *Myxomycetes* associated with dry land ecosystems of the Tehuacán-Cuicatlán Valley Biosphere Reserve, Mexico. Fungal Diversity 36: 17–56.

Lado C. (2018) An online nomenclatural information system of *Eumycetozoa*. Real Jardín Botánico, CSIC. Madrid, Spain. Available from: http://www.nomen.eumycetozoa.com (accessed 14 June 2018)

Lado C, Wrigley de Basanta D, Estrada-Torres A, García-Carvajal E. 2014. Myxomycete diversity of the Patagonian Steppe and bordering areas in Argentina. Anales del Jardín Botánico de Madrid 71(1): e006 [35 p.]. https://doi.org/10.3989/ajbm.2394

Li Y, Li HZ, Wang Q, Chen S. 1990. *Myxomycetes* from China VII: new species and new records of *Trichiaceae*. Mycosystema 3: 93–98.

Lizárraga M, Moreno G, Escobar-Zapata S. 2015. *Myxomycetes* del área urbana de Ciudad Juárez, Chihuahua, México. Boletín de la Sociedad Micológica de Madrid 39: 83–92.

Lizárraga M, Moreno G, Esqueda M. 2016. New records of myxomycetes from México. Mycotaxon 131: 511–520. https://doi.org/10.5248/131.511

Lizárraga M, Moreno G, Flores-Romero I, Jiménez-Vega F. 2018. A new species of *Physarum* (*Myxomycetes*) from Chihuahua, Mexico. Boletín de la Sociedad Micológica de Madrid 42: 21–26.

Mitchell DW, Moreno G, Lizárraga M. 2011. A new species of coprophilous *Perichaena* from New Mexico. Boletín de la Sociedad Micológica de Madrid 35: 103–108.

Moreno G, Lizárraga M, Illana C. 2007. Catálogo de los myxomycetes de México. Boletín de la Sociedad Micológica de Madrid 31: 187–229.

Moreno G, Castillo A, Deschamps JR, Giménez G, Hladki A, López-Villalba A. 2015. Critical revision of some myxomycetes deposited in the Buenos Aires Herbaria BAFC, BA and the Tucuman Herbarium LIL. IV. Boletín de la Sociedad Micológica de Madrid 39: 129–140.

Nannenga-Bremekamp NE. 1991. A guide to temperate myxomycetes. Biopress Limited, Bristol, England. 409 p.

Novozhilov YK, Schnittler M. 2001. A new coprophilous species of *Perichaena* (*Myxomycetes*) from the Russian Arctic (the Taimyr Peninsula and the Chukchi Peninsula). Karstenia 40: 117–122. https://doi.org/10.29203/ka.2000.360

Novozhilov YK, Zemlyanskaya IV, Schnittler M, Stephenson SL. 2008. Two new species of *Perichaena* (*Myxomycetes*) from arid areas of Russia and Kazakhstan. Mycologia 100(5): 816–822. https://doi.org/10.3852/08-047

Rammeloo J. 1981. Flore illustrée des champignon d'Afrique centrale. *Trichiales* (*Myxomycetes*). Fascs. 8–9. Ministère de l'Agriculture, Meise; Jardin Botanique National de Belgique.

Rojas C, Stephenson SL, Estrada-Torres A, Valverde R, Morales O. 2010. New records of myxomycetes from high-elevation areas of Mexico and Guatemala. Mycosphere 1: 73–82.

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Gliocladiopsis wuhanensis sp. nov. from China

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Abstract—A new species, *Gliocladiopsis wuhanensis*, was isolated from soil collected in Hubei Province, China. A combination of ITS, HIS3, and TUB2 DNA sequences were analyzed to evaluate its phylogenetic status. Phylogenetically *G. wuhanensis* is closely related to *G. guangdongensis*. Morphologically, *G. wuhanensis* can be distinguished from related species by its tertiary conidiophore branching.

Key words—asexual fungi, hyphomycetes, multiple gene, subtropics, taxonomy

Introduction

Gliocladiopsis S.B. Saksena was established with the type *G. sagariensis* S.B. Saksena, characterized by densely penicillate and branched conidiophores (Saksena 1954, Lombard & al. 2015). Crous & Wingfield (1993) synonymized the type species under *G. tenuis* (Bugnic.) Crous & M.J. Wingf. based on morphological studies; however, accurate identification of the *Gliocladiopsis* species based on morphology alone is difficult (Liu & Cai 2013). Through analyzing the ITS, TUB2, His3, 28S LSU, and TEF1-α DNA loci, Lombard & Crous (2012) found *G. sagariensis* and *G. tenuis* phylogenetically distinct and described five new species. Although its teleomorph was described as *Glionectria* Crous & C.L. Schoch (Schoch & al. 2000), the "anamorphic" name *Gliocladiopsis* has nomenclatural priority and is now the accepted generic name under "One Fungus: One Name". *Gliocladiopsis* currently comprises 14 species (Index Fungorum 2018) mostly soilborne (Lombard & al. 2015) except for

G. guangdongensis F. Liu & L. Cai (Liu & Cai 2013) and *G. aquaticus* Y.Z. Lu & al. (Hyde & al. 2018) isolated from decaying wood in freshwater.

Materials & methods

Isolation

Soil samples from different regions were investigated using a dilution plate method: sterilized water agar medium cooled to 45 °C was mixed with 100 µL ampicillin sodium (100 ng/µL), and poured into a sterile plate with sterile wheat-straw.; for each soil sample, three plates were evenly coated with a 200 µL soil suspension diluted 100 times, incubated ca. 3 wks at 25 °C in a biochemical incubator, and observed under stereomicroscope. Single spores were selected and transferred to PDA and synthetic nutrient-poor agar (SNA) (Nirenburg 1981) to obtain pure cultures. The fungi were examined morphologically on slides prepared with lactic acid glycerin. Measurements and descriptions of microscopic structures were made using a Nikon Eclipse 80i light microscope. One isolate was identified as representing *Gliocladiopsis,* from which more than 30 conidiogenous cells and 30 conidia were randomly selected for measurement. A dried culture specimen and living cultures were deposited in the Herbarium of Henan Agricultural University, Zhengzhou, China (HEAC).

DNA extraction, PCR amplification & sequencing

Total genomic DNA was extracted from fresh mycelia using the CTAB method (Doyle & Doyle 1990). PCR amplification primers used were: ITS region—universal primers ITS1 and ITS4 (White & al. 1990); partial β-tubulin gene—T1 (O'Donnell & Cigelnik 1997) and CYLTUB1R (Crous & al. 2004); partial Histone H3 gene—CYLH3F and CYLH3R (Crous & al. 2004). The DNA was amplified using 20 µL volumes containing 1 µL 50 ng/µL genomic DNA, 1 µL each of 10 µM primers, 10 µL Premix Ex Taq (Version 2.0, TaKaRa, containing 0.625 U DNA polymerase, 200 mM dNTP, and 1.5 mM Mg^{2+}), and 7 µL ddH_2O. The amplification protocol in an Eppendorf Mastercycler gradient thermal cycler followed an initial denaturation step of 5 min at 94 °C with 35 cycles of 45 s at 94 °C, 30 s at 58 °C, and 1min at 72 °C and a final elongation step of 10 min at 72 °C concluding with incubation at 4 °C. PCR products were examined on 1% agarose gel and sequenced on an ABI 3730XL automated DNA Analyzer by Sangon Biotech (Shanghai) Co, Ltd.

Sequence alignment & phylogenetic analyses

DNA sequences generated by each primer set were emended using SeqMan v. 7.1.0 in the DNASTAR Lasergene core suite software. Sequences were aligned and edited manually using MEGA v6.0 (Tamura & al. 2013) and then blasted in GenBank. Gene sequences of 39 strains were downloaded from NCBI, including the outgroup *Calonectria brachiatica* (CBS 123700) and *C. brassicae* (CBS 111869). The Bayesian Inference (BI) phylogenetic tree generated with MrBayes v.3.1.2 with the

Markov Chain Monte Carlo (MCMC) consisted of 4 runs (Ronquist & Huelsenbeck 2003), with each run analyzing 4 chains analyzed with 10 million generations (trees sampled every 1000 generations, a sample frequency of 100, stopping when average standard deviation of split frequencies reached below <0.01, and a burn-in of 25% (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003). Trees were viewed in FigTree v. 4.0, then modified using Adobe Illustrator CS6 and saved in Adobe Photoshop in JPG format. Newly generated sequences were deposited in GenBank.

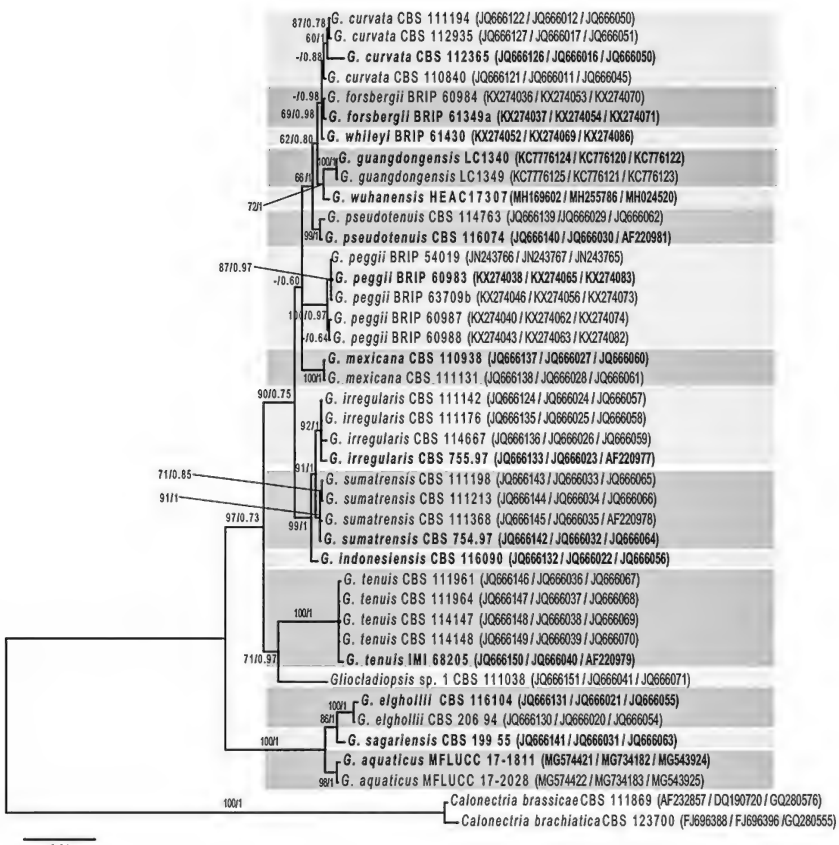

Fig. 1. Maximum-likelihood (ML) *Gliocladiopsis* tree obtained from the combined DNA sequence data from three loci (internal transcribed spacer region, histone H3, and β-tubulin). *Calonectria brachiatica* (CBS 123700) and *C. brassicae* (CBS 111869) were included as the outgroup. Bootstrap support values >50 %, and Bayesian posterior probability values >0.95, are shown at the nodes (BS/PP). Branches supported by BS = 100 % and PP = 1 are depicted as black thickened lines. Ex-type strains are in bold.

Phylogenetic results

Amplicons of approximately 400–600 base pairs were determined for TUB2 (489 bp), HIS3 (477 bp), and ITS (461 bp). The phylogenetic analysis comprised 15 ingroup and two outgroup [*Calonectria brachiatica* (CBS 123700), *C. brassicae* (CBS 111869)] taxa. The combined data set comprised 1427 characters, including 35 gaps and 775 constant characters. Analysis of the 305 parsimony-informative characters yielded one tree (Fig. 1). GTR+I+G was the model selected for Bayesian analysis and Maximum Likelihood (ML). The tree (Fig. 1) divides *Gliocladiopsis* isolates into two main clades, the first (PP = 1) containing the extype *G. sagariensis* strain (CBS 199.55), two *G. elghollii* strains (CBS 206.94, CBS 116104), and two *G. aquaticus* strains (MFLUCC 17-1811, MFLUCC 17-2028), and the second (PP = 0.73) containing the other 12 *Gliocladiopsis* species: (*G. curvata*, *G. forsbergii*, *G. guangdongensis*, *G. indonesiensis*, *G. irregularis*, *G. mexicana*, *G. peggii*, *G. pseudotenuis*, *G. sumatrensis*, *G. tenuis*, *G. whileyi*, and *G. wuhanensis*.) on the main branch.

Taxonomy

Gliocladiopsis wuhanensis Meng Zhang, N.P. Zhai & Y.H. Geng, **sp. nov.** Fig. 2

MB 825569

Differs from *Gliocladiopsis guangdongensis*, *G. curvata*, *G. forsbergii*, and *G. whileyi* by its tertiary conidiophore branching and its longer conidia.

Type: China, Hubei Province, Wuhan, from potato farm soil, 15 Jul. 2017, Niping Zhai (**Holotype**, HEAC17056 [dried culture]; ex-type living culture, HEAC17307; GenBank MH024520, MH169602, MH255786).

Etymology: *wuhanensis*, referring to the city where the type was collected.

Colonies on the SNA effuse, velvet or powdery, whitish to yellow, reverse green, reaching 6 cm diam. in two wks at 25 °C; mycelium mostly superficial or immersed, hyphae smooth, septate, branched, hyaline, 1.0–5 µm. Conidiophores penicillate, without stipe extensions and terminal vesicles, pale brown, smooth, primary branches aseptate or 1-septate, 13.0–38.5 × 2.0–4.5 µm; secondary branches aseptate, 9.5–30.5 × 2.0–4.5 µm; tertiary branches aseptate, 2.5–13.0 × 1.0–2.5 µm. Conidiogenous cells polyblastic, hyaline to light brown, 13.0–37.5 × 2.0–3.5 µm. Conidia single, smooth, hyaline, cylindrical, straight or slightly curved, 0–1-septate, 13.5–18.5 × 2.0–2.5 µm. Teleomorph unknown.

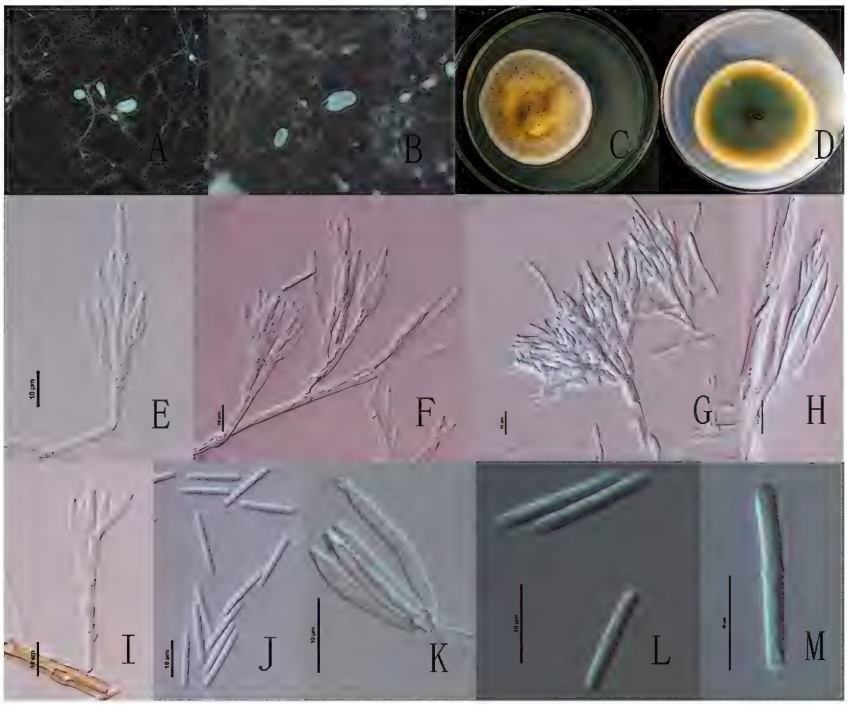

Fig. 2. *Gliocladiopsis wuhanensis* (ex-type culture). A, B. Sporulation phenotype on PDA after 10 d; C, D. Colonies on SNA after 10 d (C = top view, D = reverse view); E–I, K. Penicillate conidiophores; J, L, M. Conidia. Scale bars = 10 µm.

Discussion

Gliocladiopsis wuhanensis is morphologically similar to *G. guangdongensis*, *G. curvata* L. Lombard & Crous, *G. forsbergii* L.E. Parkinson & al., and *G. whileyi* L.E. Parkinson & al. However, *G. guangdongensis* differs by its binary conidiophore branching and its shorter conidia (13.5–16 µm; Liu & Cai 2013); *G. curvata* differs by its quaternary conidiophore branching and its shorter, wider conidia (17–21 × 3–5 µm; Lombard & Crous 2012), and *G. forsbergii* and *G. whileyi* differ by their quaternary conidiophore branching and their shorter conidia (15.5–19 µm and 17–21 µm; Parkinson & al. 2017).

Phylogenetically, *Gliocladiopsis wuhanensis* is closely related to *G. guangdongensis*. However, for the HIS3 gene, *G. wuhanensis* is separated by 12 bp from *G. guangdongensis*, greater than that from *G. forsbergii* (10 bp) and *G. whileyi* (9 bp), suggesting that they are phylogenetically more distant.

The known *Gliocladiopsis* species were isolated from different habitats (roots of diseased plants, plant litter, water, or soil) (Lombard & Crous 2012). *Gliocladiopsis* species occur quite often in soil associated with plant roots. Dann & al. (2012) conducted tests on plant roots, but the pathogenicity was not clear, and possibly they were mainly saprobic.

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Literature cited

Crous PW, Wingfield MJ. 1993. A re-evaluation of *Cylindrocladiella*, and a comparison with morphologically similar genera. Mycological Research 97: 433–448. https://doi.org/10.1016/S0953-7562(09)80131-7

Crous PW, Groenewald JZ, Risède JM, Simoneau P, Hywel-Jones NL. 2004. *Calonectria* species and their *Cylindrocladium* anamorphs: species with sphaeropedunculate vesicles. Studies in Mycology 50: 415–430. https://doi.org/10.3114/sim.55.1.213

Dann EK, Cooke AW, Forsberg LI, Pegg KG, Tan YP, Shivas RG. 2012. Pathogenicity studies in avocado with three nectriaceous fungi, *Calonectria ilicicola*, *Gliocladiopsis* sp. and *Ilyonectria liriodendri*. Plant Pathology 61: 896–902. https://doi.org/10.1111/j.1365-3059.2011.02579.x

Doyle JJ, Doyle JL. 1990. Isolation of plant DNA from fresh tissue. Focus 12: 13–15.

Huelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17: 754–755. https://doi.org/10.1093/bioinformatics/17.8.754

Hyde KD, Chaiwan N, Norphanphoun C, Boonmee S, Camporesi E & al. 2018. Mycosphere notes 169–224. Mycosphere. 9(2): 271–430. https://doi.org/10.5943/mycosphere/9/2/8

Liu F, Cai L. 2013. A novel species of *Gliocladiopsis* from freshwater habitat in China. Cryptogamie, Mycologie 34: 233–241. https://doi.org/10.7872/crym.v34.iss2.2013.233

Lombard L, Crous PW. 2012. Phylogeny and taxonomy of the genus *Gliocladiopsis*. Persoonia 28: 25–33. https://doi.org/10.3767/003158512X635056

Lombard L, van der Merwe N, Groenewald J, Crous P. 2015. Generic concepts in *Nectriaceae*. Studies in Mycology 80: 189–245. https://doi.org/10.1016/j.simyco.2014.12.002

Nirenburg HI. 1981. A simplified method to identify *Fusarium* spp. occurring on wheat. Canadian Journal of Botany 59: 1599–1609. https://doi.org/10.1139/b81-217

O'Donnell K, Cigelnik E. 1997. Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. Molecular Phylogenetics and Evolution 7: 103–116. https://doi.org/10.1006/mpev.1996.0376

Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Saksena SB. 1954. A new genus of *Moniliaceae*. Mycologia 46: 660–666. https://doi.org/10.1080/00275514.1954.12024402

Schoch CL, Crous PW, Wingfield MJ, Wingfield BD. 2000. Phylogeny of *Calonectria* and selected hypocrealean genera with cylindrical macroconidia. Studies in Mycology 45: 45–62. https://doi.org/10.3114/sim.2010.66.03

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution 30: 2725–2729. https://doi.org/10.1093/molbev/mst197

White TJ, Bruns T, Lee S, Taylor J, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. 315–322, in: MA Innis & al. (eds), PCR protocols: a guide to methods and applications. Academic Press, Orlando. https://doi.org/10.1016/b978-0-12-372180-8.50042-1

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Four new records of *Haematomma* from Southern China

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Abstract—*Haematomma accolens*, *H. collatum*, *H. fenzlianum*, and *H. flexuosum* are reported as new for China; and *H. fenzlianum* is also new for Asia. Detailed taxonomic descriptions with photos are provided for the four species.

Key words—East Asia, *Haematommataceae*, *Lecanorales*, lichenized *Ascomycota*, taxonomy

Introduction

Haematomma A. Massal., introduced by Massalongo in 1852, is a genus of crustose lichens with blood-red apothecia having thallus-coloured margins and transversely septate to muriform ascospores. For the most part, species of *Haematomma* are found in the tropics and subtropics (Staiger & Kalb 1995, Brodo & al. 2008), so it is not surprising that in China *Haematomma* is mainly distributed in the southern provinces, where the terrain is diverse with plains, basins, and plateaus, dominated by tropical subtropical monsoon climate and with high temperatures and abundant rain in summer and mild temperatures with less rain in winter. With its diverse climate types (tropical, subtropical, temperate, and cold temperate) and plant ecosystems (tropical rain forest, evergreen broadleaf forest, deciduous broadleaf forest, coniferous forest, meadow, desert, etc.), and altitudes of 72–6740 m, Yunnan Province has an especially rich range of lichen species.

[#] Rong Tang & Xiao Zhang contributed equally to this research.

In the world monograph of *Haematomma* by Staiger & Kalb (1995), only one species is mentioned as occurring in China: *H. rufidulum* (Fée) A. Massal. Previously, *H. fauriei* Zahlbr. and *H. puniceum* (Ach.) A. Massal. had been recorded from China (Zahlbruckner 1930, Xu 1989), but as we have been unable to find any specimens of these two species to verify the reports, we cannot confirm their Chinese occurrence. Since 1995, four other species have been added to the Chinese list: *H. africanum* (J. Steiner) C.W. Dodge, *H. caperaticum* Brodo & al., *H. persoonii* (Fée) A. Massal., and *H. wattii* (Stirt.) Zahlbr. (Wei 1991, Aptroot & Sparrius 2003, Obermayer 2004, Tang & al. 2018), to which we here add another four species: *H. accolens*, *H. collatum*, *H. fenzlianum*, and *H. flexuosum* based primarily on recently collected material from Southern China. Hopefully, this paper will provide the basis for further research.

Materials & methods

The specimens studied are housed in: Lichen Section of the Botanical Herbarium, Shandong Normal University (SDNU); Kunming Institute of Botany, Chinese Academy of Sciences (KUN-L); Sectio Lichenum, Herbarium Mycologici Academiae Sinicae (HMAS-L); and Lichen Herbarium of the College of Life Sciences, Liaocheng University (LCU). The macro- and micromorphological characters of the specimens were examined under an Olympus SZ51 stereomicroscope and Olympus CX21 polarizing microscope. Both thallus and medulla were tested with K (a 10% aqueous solution of potassium hydroxide) and C (a saturated solution of aqueous sodium hypochlorite). The lichen substances were identified using standardized thin layer chromatography techniques (TLC) with solvent system C (Orange & al. 2010). Photos of the voucher specimens were taken under Olympus SZX16 and BX61 with DP72.

Taxonomic descriptions

Haematomma accolens (Stirt.) Hillmann,
Repert. Spec. Nov. Regni Veg. 49: 34 (1940) Fig. 1a–e

Thallus crustose, smooth or roughened, slightly cracked, without isidia or soredia, pale yellowish white; prothallus present, white. Apothecia red, sessile or constricted at base, dispersed or crowded, smooth, epruinose, 0.4–1.0 mm diam., margin distinct, smooth; epihymenium orange-red, K+ purple, dissipating; hymenium hyaline, 75–90 µm tall; hypothecium hyaline; asci clavate, 8-spored; ascospores persistently colorless, parallel in ascus, several-septate, fusiform, with 7–12 cells in optical view, 38–60 × 3–4(–5) µm. Pycnidia: not observed.

Chemistry—Cortex and medulla K+ yellow, C–, KC–, IKI–. Atranorin, placodiolic acid, and haematommone detected by TLC.

Habitat—Growing on the bark of shrubs trees (e.g., *Betula* and *Michelia*) at elevations of 400–1230 m and in China usually growing together with *Phaeographis* spp..

Distribution—North, Central, and South America, Africa, Australia, Asia (Staiger & Kalb 1995, Brodo & al. 2008). New to China.

Specimens examined: **CHINA. Guangdong, Shixing**, Lulang, Che eight Ling National Nature Reserve, alt. 400 m, on bark, 14 May 2015, Zefeng Jia GD15156 (LCU); **Yunnan, Dali Co.**, Nanjian, Gonglang Village, Yangjiehe, alt. 1230 m, on bark, 15 Jun. 2012, Wang Li-song, Wang Xin-yu & Liu Dong 12–34410A, 12–34410C (KUN-L).

Comments—*Haematomma accolens* is characterized by the 7–12-celled ascospores and K+ purple epihymenium. The description of the Chinese material is similar to previously published descriptions of *H. accolens*, although Brodo & al. (2008) reported the spores with (5–)6–8(–12) cells. *Haematomma accolens* is morphologically similar to *H. flexuosum*, but they have a different chemistry: *H. accolens* contains atranorin, placodiolic acid, and haematommone, whereas *H. flexuosum* contains atranorin, isoplacodiolic acid, isopseudoplacodiolic acid, and haematommone.

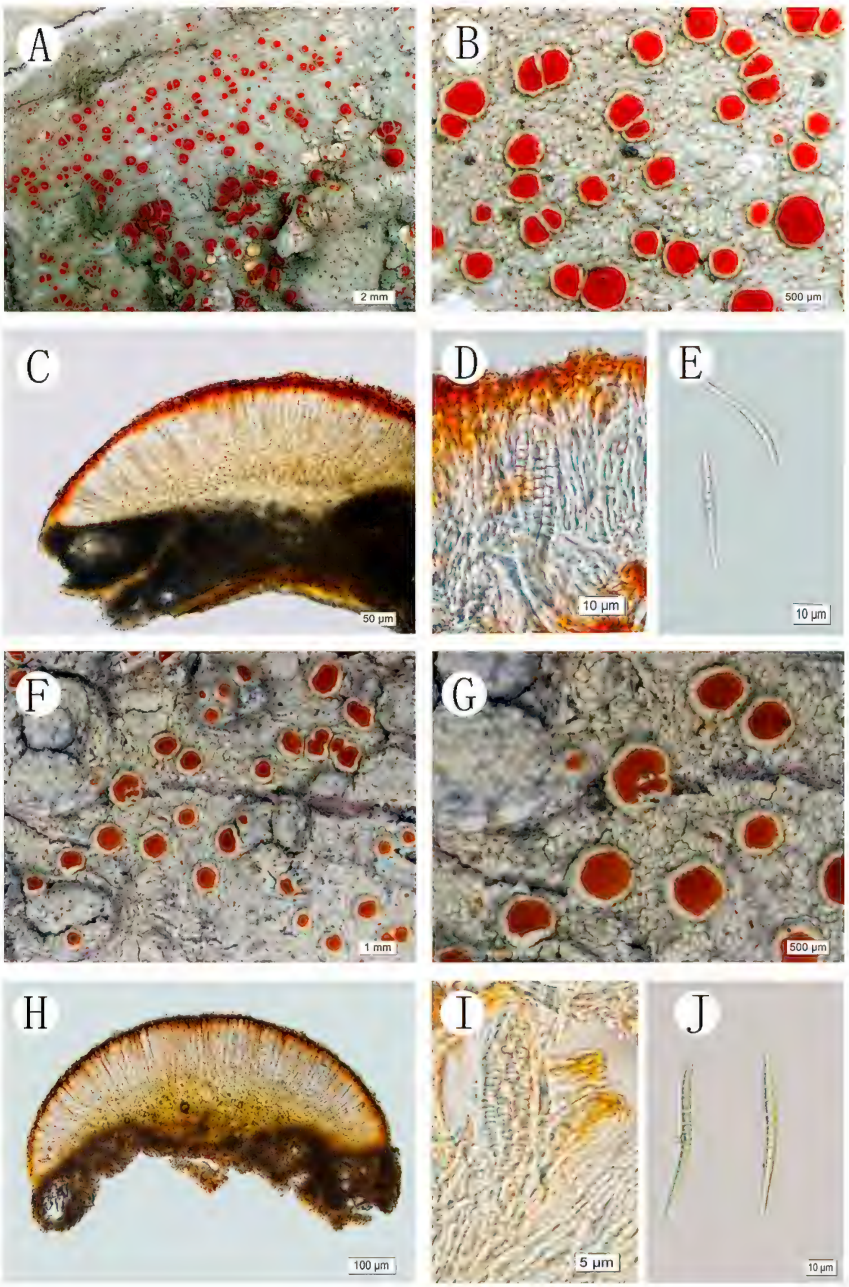
Haematomma collatum (Stirt.) C.W. Dodge,
Beih. Nova Hedwigia 38: 41 (1971) Fig. 1F–J

Thallus crustose, continuous, rugose or rimose, without isidia or soredia, yellowish white; prothallus usually present, white. Apothecia rounded, orange-red, sessile or constricted at base, dispersed, smooth, (0.5–)0.8–1.5 mm diam., margin distinct, crenulate, usually prominent above disk; epihymenium orange to orange-red, K+ red; hymenium hyaline, 80–95 µm tall; hypothecium hyaline or slightly yellowish, K–; paraphyses branched and anastomosing; asci clavate, 8-spored; ascospores persistently colorless, twisted in ascus, multiseptate, fusiform, curved, with 10–14 cells in optical view, locules sometimes not equal in size, the tail cell longer than middle cells, (53–)65–75 × 4–5 µm. Pycnidia: not observed.

Chemistry—Cortex and medulla K+ yellow, C–, KC–, IKI–. Atranorin, sphaerophorin, isosphaeric acid (minor), and russulone detected by TLC.

Habitat—Growing on bark at elevations of 670–2500 m. The specimen from Yunnan was found in a moist evergreen broadleaf forest; compared with other *Haematomma* spp. in this area, *H. collatum* grows very well. We also find that, in China, *H. collatum* likes to grow in the same environment as lichens in the *Graphidaceae*.

Distribution—Central and South America, Africa, Australia, Asia Minor (Staiger & Kalb 1995; Brodo & al. 2008). New to China.


A
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50 µm
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10 µm
E
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H
100 µm
I
5 µm
J
10 µm

Specimens examined: **CHINA. Yunnan, Jingdong,** Mt. Ailao, 24°32′29″N 101°01′37″E, alt. 2500 m, on bark, 5 Aug. 2017, R. Tang & al. 20170319 (SDNU); **Hainan, Mt. Wuzhi**, 18°46′N 109°31′E, alt. 670 m, on bark, 28 Jun. 2008, Jia Zefeng. 119392 (HMAS-L).

Comments—*Haematomma collatum* can be characterized by its crenulate apothecial margins, 10–14-celled ascospores, medullary sphaerophorin, and epithecial russulone. The Chinese material closely matches previously published descriptions (Staiger & Kalb 1995). This species and *H. persoonii* contain the same chemical substances, but *H. persoonii* has ascospores with fewer (6–8) cells (Brodo & al. 2008).

Haematomma fenzlianum A. Massal.,
Mem. Reale Ist. Veneto Sci. 10: 58 (1861) Fig. 2A–E

Thallus thick, crustose, rugose or rimose, areolate, without isidia or soredia, dull yellowish white; prothallus not seen. Apothecia rounded, deep reddish, sessile or constricted at base, dispersed, smooth, epruinose, 0.5(–0.8)–2.0 mm diam., margin distinct, crenulate or flexuose, usually prominent above disk; epihymenium orange-red, K+ red; hymenium hyaline, 70–75 µm tall; hypothecium hyaline or pale yellowish; asci clavate, 8-spored; ascospores persistently colorless, multiseptate, fusiform, straight or slightly curved, with (3–)4–7 cells in optical view, (30–)33–43(–48) × 4.5–5 µm. Pycnidia: not observed.

Chemistry—Cortex and medulla K+ yellow, C–, KC–. Atranorin, sphaerophorin, isosphaeric acid, and russulone detected by TLC.

Distribution—Europe, Africa, Australasia, America (Staiger & Kalb 1995, Nash & al. 2004, Brodo & al. 2008). New to China, and new to Asia.

Habitat—Growing on non-calcareous rock. *Haematomma fenzlianum* has rarely been collected in China, probably because it likes to grow in dry exposed areas, such as those occurring in North America and Argentina; comparable habitats are rare in southern China.

Specimen examined: **CHINA. Yunnan, Lijiang**, Yupang, alt. 2600 m, on rock, 15 Aug. 1982, Wang Li-song 82–1081 (KUN-L).

Comments—*Haematomma fenzlianum* is characterized by its thick thallus and 4–7-celled ascospores, and by its substrate. The Chinese material

Fig. 1. ***Haematomma accolens*** (LCU-L Jia GD15156). A. Thallus; B. Apothecium; C. Apothecium section; D. Ascus; E. Ascospores. ***Haematomma collatum*** (HMAS-L Jia 119392). F. Thallus; G. Apothecium; H. Apothecium section; I. Ascus; J. Ascospores.

closely matches previously published descriptions (Staiger & Kalb 1995). *Haematomma fenzlianum* is the only saxicolous species of *Haematomma* in China. *Haematomma ochroleucum* (Neck.) J.R. Laundon is also saxicolous but differs in containing zeorin and being abundantly sorediate (vs. *H. fenzlianum* which lacks zeorin and (usually) soredia).

Haematomma flexuosum Hillmann,
Repert. Spec. Nov. Regni Veg. 49: 35 (1940) FIG. 2F–J

Thallus crustose, continuous, rugose or rimose, roughened, without isidia or soredia, yellowish white; prothallus present, white. Apothecia rounded, deep reddish, sessile or constricted at base, dispersed, smooth, 0.4–0.8 mm diam., margin distinct, even with disk; epihymenium red, K+ purple, dissipating; hymenium hyaline, 63–70 µm tall; hypothecium hyaline or pale yellowish, K–; paraphyses branched and anastomosing; asci clavate, 8-spored; ascospores persistently colorless, multiseptate, fusiform, curved or straight, with 5–7 cells in optical view, (35–)50–63 × 4–5 µm. Pycnidia: not observed.

CHEMISTRY—Cortex and medulla K+ yellow, C–, KC–. Atranorin, isoplacodiolic acid, isopseudoplacodiolic acid, and haematommone detected by TLC.

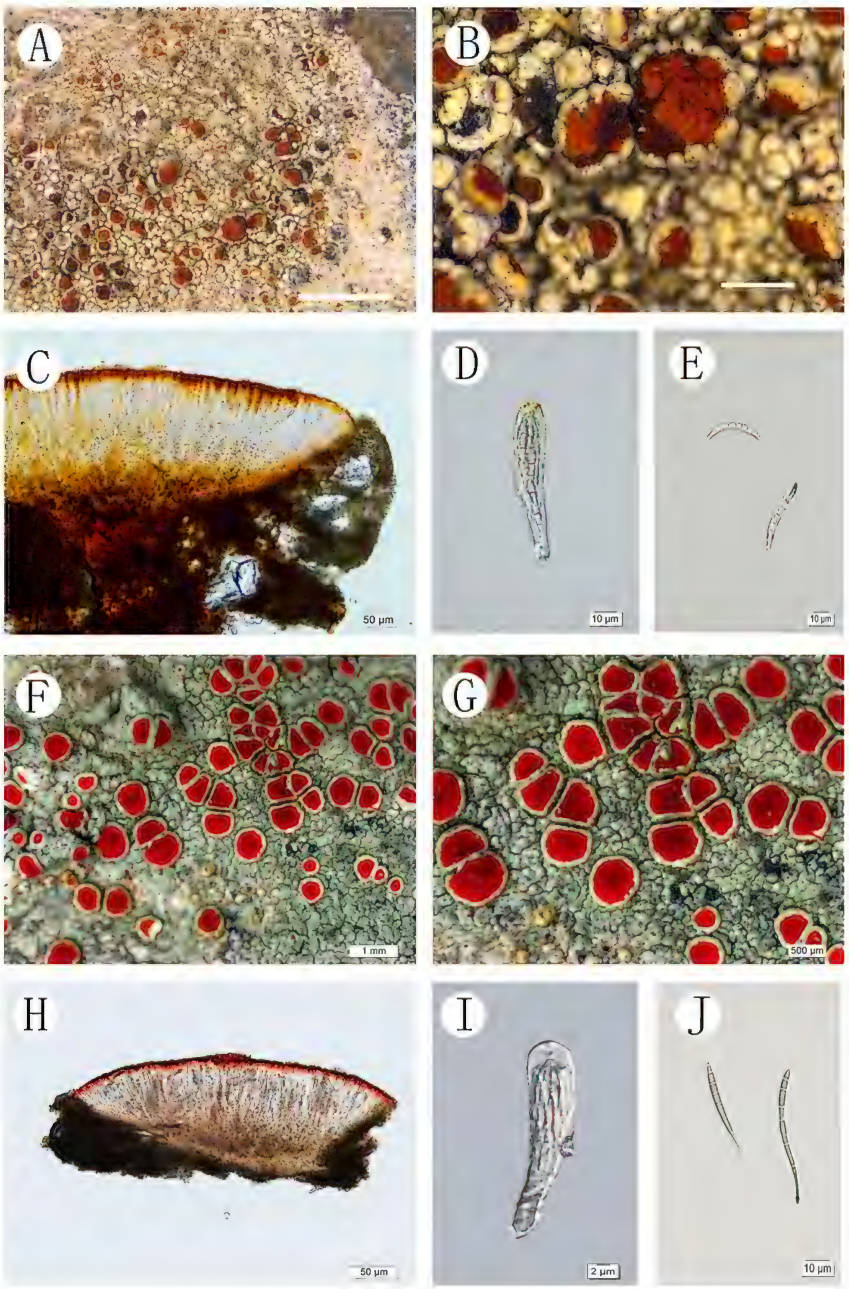
DISTRIBUTION—Central and South America, Africa, Australia, West Indies (Staiger & Kalb 1995; Brodo & al. 2008). New to China.

HABITAT—Growing on bark around 1000 m above sea level. We found this species growing together with *H. rufidulum*.

SPECIMENS EXAMINED: **CHINA. YUNNAN, Najian**, Mt. Manhai, 24°42′57″N 100°25′17″E, alt. 1266 m, on bark, 30 Jun. 2015, X. Ye & W.C. Wang 15–47956(A), 15–47956(D) (KUN-L). **YUNNAN, Luxi**, Mt. Santai, 24°26′N 98°35′E, alt. 1340 m, on bark, 28 Jun. 1980, Jiang Yu-mei 051540 (HMAS-L).

COMMENTS—*Haematomma flexuosum* is characterized by 5–7-celled ascospores and by its special chemistry. The Chinese material closely matches the description by Staiger & Kalb (1995). *Haematomma matogrossense* Kalb & Staiger is similar but differs by having 8–14-celled spores and by containing methylisopseudoplacodiolic acid and isopseudoplacodiolic acid (rather than 5–7 cells and isoplacodiolic acid and isopseudoplacodiolic acid in *H. flexuosum*).

FIG. 2. ***Haematomma fenzlianum*** (KUN-L Wang 82-1081). A. Thallus; B. Apothecium; C. Apothecium section; D. Ascus; E. Ascospores. ***Haematomma flexuosum*** (KUN-L Ye 15–47956(D)). F. Thallus; G. Apothecium; H. Apothecium section; I. Ascus; J. Ascospores. Scale bars: A = 5 mm; B = 1 mm.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C
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J
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Literature cited

Aptroot A, Sparrius LB. 2003. New microlichens from Taiwan. Fungal Diversity 14: 1–50.

Brodo IM, Culberson, WL, Culberson, CF. 2008. *Haematomma* (*Lecanoraceae*) in North and Central America, including the West Indies. Bryologist 111: 363–423. https://doi.org/10.1639/0007-2745(2008)111[363:hlinac]2.0.co;2

Nash TH, Ryan BD, Diederich P, Gries C, Bungartz F. 2004. Lichen flora of the Greater Sonoran Desert Region, vol. 2. Tempe, Lichens Unlimited.

Obermayer W. 2004. Additions to the lichen flora of the Tibetan region. Bibliotheca Lichenologica 88: 479–526.

Orange A, James PW, White FJ. 2010. Microchemical methods for the identification of lichens. 2nd edition. London, British Lichen Society.

Staiger B, Kalb K. 1995. *Haematomma*-Studien: I. Die Flechtengattung *Haematomma*. Bibliotheca Lichenologica 59. 198 p.

Tang R, Yan SK, Sun MJ, Zhang LL. 2018. New records of *Haematomma* and *Ophioparma* from China. Mycotaxon 133: 175–181. https://doi.org/10.5248/133.175

Wei JC. 1991. An enumeration of lichens in China. Beijing, China. International Academic Publishers.

Xu BS. 1989. Cryptogamic flora of the Yangtze Delta and adjacent regions. Shanghai Scientific and Technical Publishers: 158–266.

Zahlbruckner A. 1930. Lichenes. Symbolae Sinicae, vol. 3. 254 p.

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Cordana meilingensis and *C. lushanensis* spp. nov. from Jiangxi, China

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Abstract—Two new anamorphic fungi, *Cordana meilingensis* and *C. lushanensis,* were collected from dead branches in Jiangxi Province, China. *Cordana meilingensis* is characterized by its oblong or cylindrical, medially 1-septate, brown, smooth conidia with a prominent hilum. *Cordana lushanensis* is distinguished by its ellipsoidal to obovoid, pale brown, aseptate, smooth conidia with a prominent basal scar. They are described, illustrated, and compared with similar taxa.

Key words—asexual fungi, *Cordanaceae*, *Cordanales,* hyphomycetes, taxonomy

Introduction

Preuss (1851) established *Cordana* with three species, without designating a type species, and subsequently added a fourth species (Preuss 1853). However, Saccardo (1886) redescribed *Cordana*, retaining only one species, *C. pauciseptata* Preuss, which thus effectively became the type species (Hughes 1958, Seifert & al. 2011; epitypified by Hernández-Restrepo &

al. 2014). *Cordana* is mainly characterized by distinct, simple or sparely branched conidiophores, and solitary, acropleurogenous, 0–1-septate conidia seceding schizolytically from polyblastic, integrated, terminal and becoming also intercalary, sympodial conidiogenous cells with small denticles, the conidia are ellipsoidal, ovoid, obovoid, pyriform or cylindrical, often with a prominent hilum (Ellis 1971, Markovskaja 2003). Castañeda-Ruíz & al. (1999) reviewed 17 species of *Cordana* and provided a comparative table that distinguished 11 accepted *Cordana* species. However, Markovskaja (2003) regarded *C. miniumbonata* R.F. Castañeda & al. as a problematic species due to its conidial shape and septation mode. *Cordana* currently contains 19 recognized species (Hernández-Restrepo & al. 2014), distinguished primarily by conidial features including shape, size, septation, pigmentation, ornamentation, and presence or absence of a synanamorph (Castañeda-Ruíz & al. 1999, Markovskaja 2003, Hernández-Restrepo & al. 2014).

During our continuing survey (2005–18) of microfungi from plant debris in the forests of southern China, two species referable to the genus *Cordana* were collected on dead branches in Jiangxi Province. A close examination of the two fungi showed that they have significant differences from previously described *Cordana* species and are therefore proposed as new to science.

Cordana meilingensis C.C. Ai, Jian Ma, X.G. Zhang & R.F. Castañeda, **sp. nov.** Fig. 1
IF 555813

Differs from *Cordana johnstonii*, *C. uniseptata*, and *C. versicolor* by its smaller oblong or cylindrical, medially 1-septate, concolorous conidia; and from *C. mercadoana* by its larger, medially 1-septate, brown conidia.

Type: China, Jiangxi Province: Meiling National Park, on dead branches of an unidentified broadleaf tree, 8 October 2013, J. Ma (**Holotype**, HJAUP M0144).

Etymology: refers to the locality where the type specimen was found.

Colonies on the natural substratum effuse, brown to dark brown. Mycelium partly superficial, partly immersed, composed of branched, septate, smooth, subhyaline to pale brown hyphae. Conidiophores macronematous, mononematous, unbranched, erect, straight or flexuous, cylindrical, smooth, septate, brown to dark brown, paler towards the apex, 73–185 × 4–5 µm, occasionally swollen at the base, 5.5–9 µm diam. Conidiogenous cells polyblastic, integrated, terminal and intercalary, with subhyaline and slightly prominent scars, cylindrical. Conidial secession schizolytic. Conidia solitary, acropleurogenous, dry, medially 1-septate, often slightly constricted at the septa, oblong or cylindrical, brown, smooth, 10–13 × 5.5–7 µm, with a prominent hilum, 0.5–1 × 0.5 µm.

Fig. 1. *Cordana meilingensis* (holotype, HJAUP M0144).
A. Conidia; B–F. Conidiophore with conidia.

Comments—*Cordana meilingensis* resembles *C. johnstonii* M.B. Ellis, *C. uniseptata* L. Cai & al., *C. versicolor* D.J. Soares & R.W. Barreto, and *C. mercadoana* Hern.-Restr. & al. in conidial shape (Table 1). However, *C. johnstonii* has larger broadly ellipsoidal conidia and grows only on plants in the genus *Musa* (Ellis 1971); *C. uniseptata* produces larger broadly ellipsoidal, asymmetrically 1-septate, versicolored conidia (Cai & al. 2004); *C. versicolor* differs by its larger broadly ellipsoidal conidia with a paler basal cell and is parasitic on *Canna denudata* Roscoe [= *C. paniculata* Ruiz & Pav.]

(Soares & al. 2005); and *C. mercadoana* differs by its smaller, versicolored, 0–1-septate conidia (Hernández-Restrepo & al. 2014).

Table 1. Comparisons of conidia and substrates of *Cordana meilingensis* and similar species

Species	Conidia				Substrate
	Shape	Size (µm)	Color	Septation	
C. johnstonii[1]	Broadly ellipsoidal	20–30 × 12–18	Uniform	Medially 1-septate	Parasitic on *Musa* spp.
C. meilingensis	Oblong or cylindrical	10–13 × 5.5–7	Uniform	Medially 1-septate	Saprobic on dead branches
C. mercadoana[2]	Oblong, obovoid, or cylindrical	6–10 × 3–4	Variable	0–1-septate	Saprobic on dead twig
C. uniseptata[3]	Broadly ellipsoidal	13.5–23 × 8.5–11.5	Variable	Asymmetrically 1-septate	Saprobic on bamboo
C. versicolor[4]	Broadly ellipsoidal	15–25 × 10–15	Variable	Medially 1-septate	Parasitic on *Canna denudata*

Data from [1] Ellis (1971); [2] Hernández-Restrepo & al. (2014); [3] Cai & al. (2004); [4] Soares & al. (2005)

Cordana lushanensis C.C. Ai, Jian Ma, X.G. Zhang & R.F. Castañeda, **sp. nov.** Fig. 2

IF 555814

Differs from *Cordana verruculosa* by its larger smooth conidia; from *C. semaniae* and *C. solitaria* by its smaller ellipsoidal to obovoid, pale brown conidia with a prominent basal scar; and further from *C. solitaria* by lacking a *Bispora*-like synanamorph.

Type: China, Jiangxi Province: Lushan (Mount Lu), on dead branches of an unidentified broadleaf tree, 8 November 2017, J. Ma (**Holotype**, HJAUP M5406).

Etymology: refers to the locality where the type specimen was found.

Colonies on the natural substratum effuse, brown to dark brown. Mycelium partly superficial, partly immersed, composed of branched, septate, smooth, subhyaline to pale brown hyphae. Conidiophores macronematous, mononematous, simple or branched, erect, straight to flexuous, cylindrical, with intercalary nodes, 7–14 µm diam, brown, paler toward the apex, smooth, ≤240 × 6.5–7.5 µm. Conidiogenous cells integrated, polyblastic, terminal and intercalary, with subhyaline small denticles, proliferations percurrent, cylindrical to lageniform. Conidial secession schizolytic. Conidia solitary, acropleurogenous, dry, 0-septate, ellipsoidal to obovoid, pale brown, smooth, 5.5–8 × 2.5–4 µm, with a prominent basal scar, 0.3–0.5 µm diam.

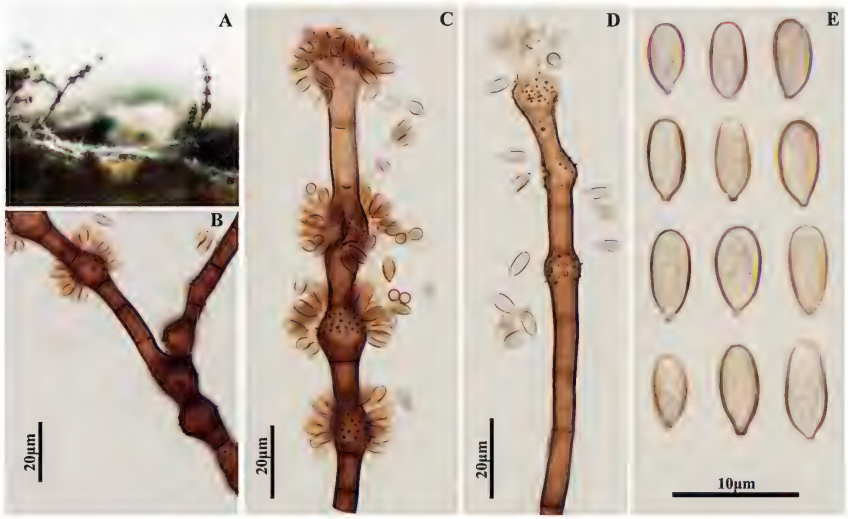

Fig. 2. *Cordana lushanensis* (holotype, HJAUP M5406).
A. Colonies on natural substratum; B–D. Conidiophores and conidia; E. Conidia.

Comments – Among the known species, only *C. semaniae* Davydkina & al., *C. solitaria* V. Rao & de Hoog, and *C. verruculosa* Hern.-Restr. & al. resemble *C. lushanensis* in producing aseptate conidia. However, *C. verruculosa* differs by its smaller (3–5.5 × 2–3.5 μm) verruculose conidia (Hernández-Restrepo & al. 2014); *C. semaniae* differs by its larger (21–27 × 9–15 μm) obovoid black conidia with an acute basal cell (Davydkina & Mel'nik 1989); *C. solitaria* differs by its broader (4.5–6.5 μm diam) obovoid conidia with a slightly papillate base, and the presence of a *Bispora*-like synanamorph (Rao & de Hoog 1986).

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Literature cited

Cai L, McKenzie EHC, Hyde KD. 2004. New species of *Cordana* and *Spadicoides* from decaying bamboo culms in China. Sydowia 56(2): 222–228.

Castañeda-Ruíz RF, Iturriaga T, Guarro J. 1999. A new species of *Cordana* from Venezuela. Mycotaxon 73: 1–8.

Davydkina TA, Mel'nik VA. 1989. Two new hyphomycetes from the genera *Cordana* and *Pyriculariopsis*. Mikologiya i Fitopatologiya 23(2): 110–113.

Ellis MB. 1971. Dematiaceous hyphomycetes. X. Mycological Papers 125. 30 p.

Hernández-Restrepo M, Gené J, Mena-Portales J, Cano J, Madrid H, Castaneda-Ruíz RF, Guarro J. 2014. New species of *Cordana* and epitypification of the genus. Mycologia 106(4): 723–734. https://doi.org/10.3852/13-122

Hughes SJ. 1958. Revisiones hyphomycetum aliquot cum appendice de nominibus rejiciendis. Canadian Journal of Botany 36: 727–836.

Markovskaja S. 2003. A new species of *Cordana* from Lithuania. Mycotaxon 87: 179–185.

Preuss CGT. 1851. Übersicht untersuchter Pilze, besonders aus der Umgegend von Hoyerswerda. Linnaea 24: 99–153.

Preuss CGT. 1853. Übersicht untersuchter Pilze, besonders aus der Umgegend von Hoyerswerda. Linnaea 25: 723–742.

Rao VG, de Hoog GS. 1986. New or critical hyphomycetes from India. Studies in Mycology 28. 84 p.

Saccardo PA. 1886. Sylloge hyphomycetum. Sylloge Fungorum 4. 807 p.

Seifert K, Morgan-Jones G, Gams W, Kendrick B. 2011. The genera of hyphomycetes. CBS Biodiversity Series 9. 997 p.

Soares DJ, Nechet KL, Barreto RW. 2005. *Cordana versicolor* sp. nov. (dematiaceous hyphomycete) causing leaf-spot on *Canna denudata* (*Cannaceae*) in Brazil, with observations on *Cordana musae*. Fungal Diversity 18: 147–155.

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Coprophilous fungi from Brazil: new records for the Neotropics

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Abstract—Twelve ascomycetes associated with herbivore dung, collected throughout an edaphic and climatic gradient from the Atlantic Forest complex to the semi-arid Caatinga in Pernambuco, Northeastern Brazil, represent new records: *Ascobolus castaneus*, *Arnium hirtum, Cercophora anisura*, *Chaetomium citrinum, C. spirale, Coniochaeta philocoproides*, *Coprotus albidus,* and *Delitschia vulgaris* are new records for the Neotropics; *Cercophora sordarioides, Chaetomium cochliodes, Sordaria lappae*, and *Thelebolus microsporus* are new records for Brazil. *Ascobolus castaneus* is recorded for the second time worldwide, revealing a much wider geographical distribution for a species previously reported only from China. Descriptions and updated geographic distributions are presented, and information regarding the records is discussed.

Key words—*Ascomycota*, dung fungi, taxonomy

Introduction

Coprophilous fungi constitute a rich and diverse ecological group of saprobes especially adapted to withstand conditions and use resources available in herbivore dung (Kirschner & al. 2015). These fungi can be found in dung of many herbivores, domesticated or wild, all over the world and throughout the year (Richardson 2001). Fungi on dung are represented mainly by members of *Ascomycota* (e.g., *Ascobolaceae*, *Lasiosphaeriaceae*), *Mucoromycota*

(e.g., *Pilobolaceae*, *Mucoraceae*), and *Basidiomycota* (e.g., *Coprinaceae*, *Psathyrellaceae*). As in other ecological groups, a gradient ranging from specialized species to opportunistic saprobes can be found in coprophilous fungal communities (Newcombe & al. 2016). Despite this, the coprophilous mycobiota retains some similarity and shows somewhat regular patterns of occurrence and abundance throughout continents and time (Basumatary & McDonald 2017). The Brazilian coprophilous mycobiota is poorly known, and recent works have focused on the taxonomy and distribution of the most common genera and in establishing regional and national checklists (Calaça & al. 2014; Melo & al. 2012, 2017; Souza & al. 2017). As most coprophilous species are restricted to their substrates, their geographical distribution is only known where such inventories have been conducted, and some species thought to be restricted to temperate regions are being reported in the tropics as well. This work presents the first report of twelve records of *Ascomycota* associated with herbivore dung, all new records either for the Neotropics, South America, or Brazil.

Materials & methods

A total of 270 dung samples from goat, cattle, and horse were sampled between 2011 and 2013 from three animal precincts: (1) Universidade Federal Rural de Pernambuco, Veterinary Medicine Department, Recife (8°00′54″S 34°56′59″W), (2) Instituto Agronômico de Pernambuco (IPA), Caruaru (8°01′59″S 36°06′59″W), and (3) Instituto Agronômico de Pernambuco (IPA), Serra Talhada (7°54′59″S 38°17′00″W). These areas are located along a latitudinal transect of ~420 km in the state of Pernambuco, Northeastern Brazil and encompass two biomes: the Atlantic Rainforest to the east and the semiarid Caatinga to the west. Additional material, from Rio Grande do Sul state, were also received. Fresh dung samples were collected in clean plastic bags, taken to the laboratory, and incubated in moist chambers at room temperature (28 ± 2 °C) for at least 75 days under alternating natural light and dark periods. The material was observed directly on the substrata with the aid of a stereomicroscope, and ascomata (with or without their asexual morphs) were mounted in water, lactic acid, or lactophenol with cotton blue for identification under light microscopy.

Species were identified according to Ames (1961), Brummelen (1967), Kimbrough & al. (1972), von Arx & al. (1986), Bell (1983, 2005), Doveri (2004), Lundqvist (1972), and Richardson & Watling (1997). Careful review of the literature and national database searches were conducted to access information regarding previous records of coprophilous ascomycetes in Brazil. All voucher material was deposited at the Herbarium Padre Camille Torrend, Departamento de Micologia, Universidade Federal de Pernambuco, Recife, Brazil (URM). Additional information regarding all records and deposited specimens, along with high quality micrographs,

are available at the INCT - Herbário Virtual da Flora e dos Fungos database website (http://inct.florabrasil.net).

Taxonomy

Dothideomycetes, Pleosporales, Delitschiaceae

Delitschia vulgaris Griffiths, Mem. Torrey Bot. Club 11: 104 (1901) Fig. 1A–C

Pseudothecia immersed, scattered, obpyriform, dark brown to black, 1275–1450 × 350–460 µm, with body adorned with light brown to golden, thin, flexuous, simple or branched hyphoid hairs with a slightly thickened wall, septate, about 1.5–2 µm diam., ≤150 µm long; neck long, cylindrical, black, with papilliform rounded apex, 690–725 × 165–210 µm, tomentose; Asci 8-spored, cylindrical, fissitunicate, uniseriate, 275–300 × 20–25.5 µm, with rounded apices and short stipe; Ascospores 2-celled, with a medial septum, oblong-ellipsoid to fusoid, weakly constricted, with slightly narrowed ends, light brown when young, becoming dark brown at maturity, smooth, 32.5–37.5 × 13–15 µm; germ slits longitudinal, spanning the entire length of each cell.

Specimens examined—**BRAZIL. Pernambcuo: Recife,** Universidade Federal Rural de Pernambuco (UFRPE), on horse dung, 13.III.2012, R.F.R. Melo s.n. (URM86772a, b, c).

Distribution—Europe (Sweden), North America (Canada, USA), Oceania (Australia). This is the first record for the Neotropics.

Comments—The *Delitschia vulgaris* material from Brazil presents larger ascospores compared to the material cited by Luck-Allen & Cain (1975). Considering *Delitschia* species with tomentose pseudothecia and ascospores with a transverse septum, *D. araneosa* Cain is similar to *D. vulgaris*, but differs by its slightly narrower ascospores (10–11 µm; Luck-Allen & Cain 1975).

Leotiomycetes, Thelebolales, Thelebolaceae

Coprotus albidus (Boud.) Kimbr., Amer. J. Bot. 54: 22 (1967) Fig. 1D, E

Apothecia scattered, globose to lenticular, subhyaline to creamy-yellow, translucent, 300–600 µm diam., sessile, glabrous; margin rounded, entire and smooth; disk concolorous with the receptacle, scurfy to slightly villose due to the ascal protrusions; medullary excipulum with intricate cells (textura intricata); ectal excipulum composed of elongated cells, 7.5–10 µm diam.; Paraphyses cylindrical, becoming clavate towards the apex, enlarged to 5–6.5 µm wide, unbranched, septate, without visible refractive globules or pigments; Asci 32-spored, clavate, with ascospores irregularly disposed, 80–110 × 22.5–30 µm, with a rounded apex; Ascospores 1-celled, ellipsoid

to elongated-ellipsoid, with tapered ends, hyaline, smooth, (7.5–)10–12.5 × 5–7.5(–10) µm, mostly with a de Bary bubble.

Specimen examined—**BRAZIL. Rio Grande du Sol: Santana do Livramento,** Área de Proteção Ambiental do Ibirapuitã, on horse dung, 13.VI.2015, Xavier de Lima s.n. (URM92164).

Distribution—Europe (Denmark, England, Finland, France). This is the first record for the Neotropics.

Comments—The material described here matches closely *C. albidus* as described by Kimbrough & al. (1972). Among *Coprotus* species with whitish to translucent apothecia bearing 32-spored asci and growing on dung, *C. albidus* can be confused with *C. rhyparobioides* (Heimerl) Kimbr., which presents larger asci (120–175 × 50–75 µm) and longer ascospores (13.5–17.5 × 7–8 µm), as well as paraphyses frequently branched toward apices (Kimbrough & al. 1972).

Thelebolus microsporus (Berk. & Broome) Kimbr.,
Ann. Rep, Inst. Ferment. Res. Osaka: 50 (1967) Fig. 1F, G

Apothecia scattered to gregarious, with cleistohymenial development pattern, initially globose to obpyriform, becoming cupulate to lenticular, exposing the disk later in the telohymenial phase, white to pale yellow, becoming yellowish to pale luteous, 95–250 µm diam., sessile, glabrous; margin rounded, entire and smooth; disk concolorous with the receptacle, almost smooth; hymenium ~45–100 µm thick; medullary excipulum and hypothecium undifferentiated; ectal excipulum composed of intertwined thick hyphae (textura epidermoidea), 7.5–12.5 µm diam.; Paraphyses cylindrical, branched, septate, 2–3 µm wide at the base, without visible refractive globules or pigments, branched close to its base and conspicuously broadened towards apices, enlarged up to 6 µm diam.; Asci 8-spored, cylindric-clavate, irregularly biseriate or uniseriate, 80–95 × 10–25 µm, with a short stipe, rounded above; Ascospores 1-celled, ellipsoid, with rounded ends, hyaline, smooth, 7.5–10 × 3–5 µm, occasionally with a de Bary bubble close to one end (absent when mounted in water).

Specimens examined—**BRAZIL. Pernambuco: Caruaru,** Instituto Agronômico de Pernambuco (IPA), on cattle dung, 16.X.2011, R.F.R. Melo s.n. (URM86783); on horse dung, 13.VI.2012, R.F.R. Melo s.n. (URM86784); **Serra Talhada,** Instituto Agronômico de Pernambuco (IPA), on goat dung, 21.IX.2011, R.F.R. Melo s.n. (URM86785).

Distribution—Antarctica, Europe (Austria, Denmark, England, Estonia, Faroe Islands, France, Germany, Ireland, Netherlands, Norway, Sweden, Switzerland, Ukraine), North America (Canada USA), Oceania (Australia, New Zealand), South America (Argentina). This is the first record for Brazil.

Comments—Some measurements of the Brazilian material of *Thelebolus microsporus*, such as ascospore length and width, showed slight variation compared with material cultivated by Hoog & al. (2005) from Antarctica. This species differs from other 8-spored members of *Thelebolus* mainly by its developmental pattern: the apothecia expose their asci late in the mesohymenial or telohymenial phase, resulting in a typical apothecioid morphology, in contrast with species such as *T. globosus* Brumm. & de Hoog, which forms globose apothecia.

Pezizomycetes, Pezizales, Ascobolaceae

Ascobolus castaneus Teng, Sinensia 11: 109 (1940) Fig. 1H–J

Apothecia usually gregarious, with eugymnohymenial development pattern, initially globose to subglobose, becoming scutellate to discoid when mature, at first yellowish-brown, later chestnut brown, drying rust brown to finally blackish, 0.8–2 mm diam., sessile, almost glabrous; margin rounded, smooth, bending outwards with age; disk at first concave and yellow in maturation, finally convex, concolorous with the receptacle, smooth to slightly villose, not furfuraceous; hypothecium 30–47.5 µm thick, mostly with strongly compact, isodiametric cells (textura angularis); ectal excipulum composed of irregular ellipsoid cells, becoming more globose towards the margins (textura globosa near the margin, textura angularis near the base), 30–50 µm wide; Paraphyses cylindrical, branched, septate, 2–3 µm diam. at the base, with brownish intercellular pigment, slightly to not enlarged at the apex; Asci 8-spored, cylindric-clavate, irregularly biseriate or uniseriate, 120–175 × 15–17.5 µm, with a short stipe, rounded above; Ascospores 1-celled, ellipsoid, with rounded ends, at first hyaline, then brown to reddish brown, smooth, 21.5–26.5 × 11.5–12.5 µm.

Specimen examined—**BRAZIL. Pernambuco: Recife,** Universidade Federal de Pernambuco, on horse dung, 06.VI.2017, D.B.P. Monte s.n. (URM92165).

Distribution—Asia (China). This is the first record for the Neotropics.

Comments—*Ascobolus castaneus* is distinguished by its chestnut brown mature apothecia and completely smooth ascospores. It can be confused with other common members of *A.* sect. *Gymnascobolus* Brumm., such as *A. scatigenus* (Berk. & M.A. Curtis) Brumm., when young—both present similar apothecia up to the mesohymenial phase but can be immediately distinguished when mature. *Ascobolus scatigenus* has relatively large receptacles, white to pale luteous, furfuraceous disks composed of protruding asci with ellipsoid

ascospores (22–24.5 × 10–15 µm according Melo & al. 2014), usually with a single to few oblique epispore fissures. The material was collected in situ on horse dung and not observed in moist chamber incubation like the others mentioned in this work. The ascospores are smaller than those in the Chinese material described by Van Brummelen (1972; 15–19.5 × 7.5–9 µm).

Sordariomycetes, Coniochaetales, Coniochaetaceae

Coniochaeta philocoproides (Griffiths) Cain,
Univ. Toronto Stud., Biol. Ser. 38: 65 (1934) FIG. 1K–O

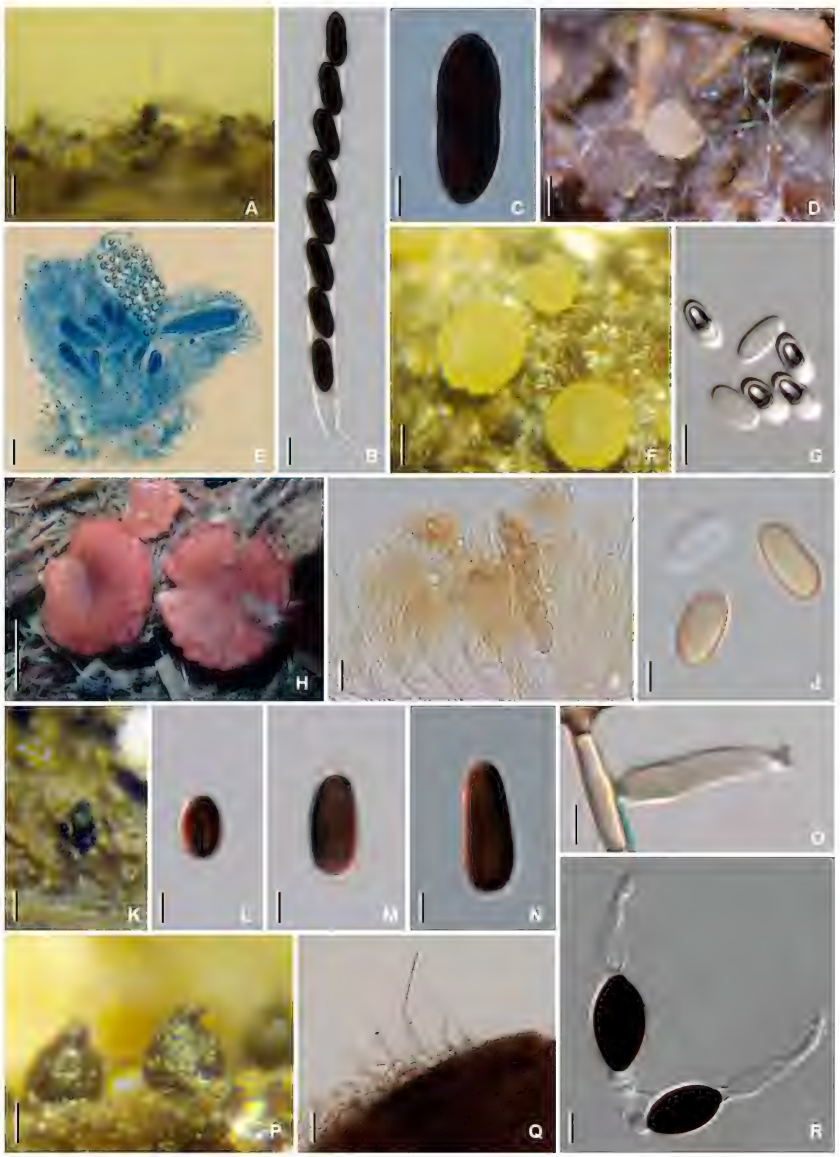
PERITHECIA scattered to gregarious, partially immersed, subglobose to obpyriform, brown to dark brown, 325–410 × 310–390 µm; NECK short, papilliform, occasionally indistinct; PERIDIUM membranaceous, opaque, dark brown, becoming dark brown to finally black towards the neck; HAIRS dark brown to black, rigid, usually straight, simple, thick-walled, non-septate, becoming thinner at the apex, abundant at the neck; ASCI 32-spored, cylindric to cylindric-clavate, irregularly triseriate to tetraseriate, 112–120 × 14–20 µm, with a short stipe, rounded above; ASCOSPORES 1-celled, variable in length, ellipsoid to oblong in frontal view, conical in lateral view, light brown to golden when young, becoming dark brown at maturity, smooth, 7.5–12(–22) × 57.5 µm, with a lateral straight germ slit through the entire length of the spore, without conspicuous gelatinous sheath; CONIDIOPHORES hyaline, simple or branched, septate; CONIDIOGENOUS CELLS enteroblastic, hyaline, consisting of discrete phialides, ampulliform to lageniform, 10–15(–22.5) × 2.5–3 µm, with cylindrical collarettes, 1–2 µm long; CONIDIA not observed.

SPECIMEN EXAMINED—**BRAZIL. PERNAMBUCO: Caruaru,** Instituto Agronômico de Pernambuco (IPA), on horse dung, 28.II.2013, R.F.R. Melo s.n. (URM86634).

DISTRIBUTION—Europe (Norway), North America (USA), and Oceania (New Zealand). This is the first record for the Neotropics.

COMMENTS—The specimen from Brazil matches closely the material described by Asgari & al. (2007). *Coniochaeta philocoproides*, a rare species reported on herbivore dung, is one of the few documented multispored species in this genus. Asci of *C. polymegasperma* M.J. Richardson are 64-spored, and its ascospores are longer (13–16.5 µm) and larger (9.5–13.5 µm) than *C. philocoproides*.

FIGURE 1. Brazilian dung fungi: ***Delitschia vulgaris***. A. pseudothecium on dung; B. ascus; C. mature ascospore, with two visible germ slits, once at each cell. ***Coprotus albidus***. D. apothecia on dung; E. mounted apothecium, with asci in different developmental stages. ***Thelebolus microsporus***. F. apothecia on dung; G. ascospores. ***Ascobolus castaneus***. H. apothecia on dung; I. hymenium,



with asci in different developmental stages; J. ascospores. ***Coniochaeta philocoproides***. K. perithecium on dung; L, M, N. ascospores from the same ascus, considerably different in length; O. phialide. ***Arnium hirtum***. P. perithecia on dung; Q. perithecial hairs; R. mature ascospores. Scale bars: A = 750 µm; B, E = 20 µm; C, J = 10 µm; D = 500 µm; F = 100 µm; G = 7.5 µm; H = 1 mm; I = 25 µm; K = 200 µm; L–O = 5 µm; P = 400 µm; Q = 2.5 µm; R = 15 µm.

Coniochaeta hansenii (Oudem.) Cain is reported with 64–128-spored asci and slightly different ascospore measurements (6–9 × 5–9 µm) (Asgari & al. 2007).

Sordariomycetes, Sordariales, Lasiosphaeriaceae

Arnium hirtum (E.C. Hansen) N. Lundq. & J.C. Krug,
Symb. Bot. Upsal. 20(1):218 (1972) FIG. 1P–R

PERITHECIA scattered to isolated, semi-immersed to superficial, obpyriform, 690–930 × 480–540 µm, reddish brown to dark brown, translucent, covered by hyphoid, slender, flexuous hairs, septate, 2–2.5 µm thick; NECK cylindrical to conical, opaque, 150–165 × 130–140 µm, tomentose, neck hairs more rigid and setose, ≤7 µm long, becoming thinner towards the apex; PERIDIUM membranaceous, subopaque to olivaceous brown, darkening towards the neck to finally black, 3-layered—outer peridial layer composed of large, inflated, angular cells, thick-walled, 5–10 µm diam., forming a short palisade that resembles inflated perithecial hairs; PARAPHYSES interspersed with the asci, ventricose, persistent or collapsing; ASCI 8-spored, cylindric to cylindric-clavate, 350–400 × 25.5–35.5 µm, usually persistent, with rounded apex and a long stipe; ASCOSPORES at first fusoid to ellipsoid, hyaline, later becoming ochraceous to olivaceous brown, with several oil droplets, and finally dark brown, bicaudate; 35–47.5 × 18.5–22.5 µm, with an eccentric germ pore; APICAL CAUDA single, 7.5–9 µm diam., becoming thinner towards its apex, up to 42.5 µm long, usually persistent in mountings, longitudinally striped, covering the germ pore; BASAL CAUDA similar in morphology, slightly thinner and longer, 5–6 µm wide at the base when not inflated, up to 50 µm long, persistent or collapsing when mounted.

SPECIMENS EXAMINED—**BRAZIL. PERNAMBUCO: Serra Talhada,** Instituto Agronômico de Pernambuco (IPA), on goat dung, 24.X.2011, R.F.R. Melo s.n. (URM86719); , Universidade Federal Rural de Pernambuco (UFRPE), on horse dung, 23.III.2012, R.F.R. Melo s.n. (URM86720).

DISTRIBUTION—Africa (Morocco), Europe (Belgium, England, Denmark, France, Germany, Ireland, Norway, Scotland, Sweden) and North America (USA). This is the first record for the Neotropics.

COMMENTS—*Arnium* species are distinguished from other dark-coloured perithecial dung ascomycetes by their unicellular ascospores with gelatinous caudae at both ends. *Arnium hirtum* presents a wide array of morphological variation, having as its main characteristics the substrate (herbivore dung) and the ascospore morphology: equilateral, non-septate and bicaudate (35–47.5 × 18.5–22.5 µm). The present material fits well with the description of the

European specimens (Lundqvist 1972), with similar measurements and general morphology. It is a common species in Europe, but rare outside that continent, although more surveys will probably reveal new records for tropical areas.

Cercophora anisura N. Lundq., Symb. Bot. Upsal. 20(1): 91 (1972) FIG. 2A, B

PERITHECIA isolated, semi-immersed, obpyriform, subglobose or conical, 480–515 × 350–400 µm, light brown to golden, translucent, covered by hyphoid, flexuous, occasionally branched hairs, about 2 µm thick; NECK cylindrical to conical, opaque, carbonaceous, dark brown to finally black close to the apex, 100–110 × 80–100 µm, adorned with tufts of short, inflated and agglutinated hairs, 2–2.5 µm thick, septate, concolorous with the peridium in the lower part of the perithecium; PERIDIUM pseudo-parenchymatous, membranaceous, subopaque, darkening towards the neck to finally black, 3-layered—outer peridial layer composed of angular to rounded cells, thin-walled, 4–9 µm diam.; PARAPHYSES interspersed with the asci, ventricose, persistent or collapsing; ASCI 8-spored, cylindric-clavate, 175–225 × 17.5–25 µm, with a long stipe and tapered apex, thickened, simple apical ring present; subapical globule subglobose to ellipsoid, smooth to slightly verrucose, 4–5 µm diam.; ASCOSPORES at first cylindrical vermiform to slightly sigmoid, hyaline, with several oil droplets, occasionally geniculate at the base, non-septate, smooth, bicaudate, later becoming swollen in the upper part and finally 2-celled after the formation of a transverse septum, delimiting the region of the spore that will become the pigmented head cell above, and another part for the hyaline pedicel below, irregularly biseriate to triseriate; HEAD CELL ellipsoid, truncated at the base, non-septate, brown to dark brown, smooth, 15–19 × 7.5–8 µm with an eccentric germ pore; PEDICEL cylindrical, hyaline, occasionally geniculate near the base, usually collapsing, 30–35 × 4–5 µm; APICAL CAUDA single, cylindrical to conical, tapering towards the apex, 10–20 µm long, 2–2.5 µm diam. at the base, usually collapsing; BASAL CAUDA similar in morphology, thicker and longer, 20–28.5 × 3–3.5, usually collapsing.

SPECIMENS EXAMINED—**BRAZIL. PERNAMBUCO: Recife,** Universidade Federal Rural de Pernambuco (UFRPE), on horse dung, 11.XII.2012, R.F.R. Melo s.n. (URM86721a, b, c).

DISTRIBUTION—Europe (Sweden). This is the first record for the Neotropics.

COMMENTS—The Brazilian material was sparse and little information regarding its morphological variation is presented here. *Cercophora anisura* was proposed by Lundqvist (1972) in his monographic treatment of the Nordic *Sordariaceae* from material on cattle dung in Sweden. The species can be confused with *C. mirabilis* Fuckel, one of the most common *Cercophora* on herbivore dung,

which differs by its larger perithecia (650790 × 340475 µm), longer inflated hairs (2–3.5 µm), and ascospores with even caudae (Lundqvist 1972). In our material of *C. anisura*, mature ascospores with multiseptate pedicels, ascospores with both cells pigmented, and ascospores without the transverse septum were commonly observed.

Cercophora sordarioides (Speg.) N. Lundq.,
Symb. Bot. Upsal. 20(1): 112 (1972) FIG. 2C–E

PERITHECIA scattered to isolated, semi-immersed to superficial, obpyriform, subglobose or conical, 650–850 × 550–600 µm, olivaceous to golden, translucent, glabrous; NECK conical to slightly cylindrical, opaque, carbonaceous, dark brown to finally black close to the apex, 200–350 × 190–200 µm; PERIDIUM pseudo-parenchymatous, membranaceous, subopaque, darkening towards the neck to finally black, 3-layered—outer peridial layer with angular to rounded cells, thin-walled, 7–10(–12.5) µm diam.; PARAPHYSES interspersed with the asci, filiform, persistent or collapsing; ASCI 8-spored, clavate, 155.5–185 × 17.5–25 µm, with a long stipe and tapered apex, thickened, simple apical ring present; subapical globule subglobose to globose, smooth to slightly verrucose, 4–5.5 µm diam.; ASCOSPORES at first cylindrical to sigmoid, hyaline, with several oil droplets, occasionally geniculate at the base, non-septate, smooth, bicaudate, later becoming swollen in the upper part and finally 2-celled after the formation of a transverse septum, delimiting the region of the spore that will become the pigmented head cell above, and another part for the hyaline pedicel below, irregularly biseriate to triseriate; HEAD CELL ellipsoid, truncated at the base, usually with an eccentric transverse septum, brown to dark brown, smooth, 17.5–22.5 × 7.5–11 µm with a apical or subapical germ pore; PEDICEL cylindrical, hyaline, occasionally geniculate near the base, persistent or collapsing, 45.5–49 × 29.5–31 µm; APICAL CAUDA single, cylindrical, tapering towards the apex, 27.5–55 µm long, 2.5–3 µm wide at the base, usually collapsing; BASAL CAUDA similar in morphology.

SPECIMEN EXAMINED—**BRAZIL. PERNAMBUCO: Caruaru,** Instituto Agronômico de Pernambuco (IPA), Caruaru, on horse dung, 23.XI.2012, R.F.R. Melo. s.n. (URM86728).

DISTRIBUTION—Europe (Sweden). This is the first record for the Neotropics.

COMMENTS—*Cercophora sordarioides* is regarded as a rare species. It was reported from Argentina by Spegazzini. The key characteristics are the glabrous, olivaceous to dark-yellow perithecia with thin-walled peridial cells and ascospores with septate head cells. The Brazilian material presents longer pedicels in relation to Argentine material (37–38 µm), as described in Lundqvist (1972).

Sordariomycetes, Sordariales, Chaetomiaceae

Chaetomium citrinum Udagawa & T. Muroi,
Trans. Mycol. Soc. Japan 22: 15 (1981) FIG. 2F–H

PERITHECIA scattered to isolated, superficial, subglobose to obovoid, yellowish to greenish in mass, dark brown when isolated, 250–300 × 240–280 µm, attached to the substrate by weakly pigmented rhizoids, light brown to brown, thin-walled, dichotomously branched, non-septate, flexuous, usually smooth, 2.5–2.8 µm diam.; TERMINAL HAIRS of one type — numerous, septate, wavy to loosely coiled, forming distant and weakly organized coils along its length, making 2–3 convolutions, 2.4–3 µm diam., with almost constant diameter in each turn towards the apex, light brown to golden, becoming less pigmented close to the apex, extending up to 400(–450) µm, markedly punctate in all its extension, clustered above to form a strongly compact sporiferous head above the perithecium, ellipsoid to columnar; LATERAL HAIRS slightly similar in morphology — numerous, long, filiform, tortuous, smooth, sparsely septate, thin-walled, light brown to golden, slightly less pigmented than the terminal hairs, 1.9–2.4 µm, extending up to 350 µm; PERIDIUM pseudo-parenchymatous, membranaceous, fragile, with flattened, angular to elongate, slightly thick-walled cells; ASCI 8-spored, clavate, spore-bearing part 9–12.5 µm diam., with a short stipe, evanescent; ASCOSPORES variable in shape, ellipsoid, long-ellipsoid, ovoid, fusoid to limoniform, biapiculate or not, dark brown when mature, smooth or with superficial cracklings, 7.4–10.6 × 4.8–6.5 µm.

SPECIMENS EXAMINED—**BRAZIL. PERNAMBUCO: Caruaru,** Instituto de Pesquisas Agronômicas, on goat dung: 27.II.2012, R.F.R. Melo s.n. (URM86708), 25.VI.2012, R.F.R. Melo s.n. (URM86709a, 86709b), **Recife,** Universidade Federal Rural de Pernambuco (UFRPE), on goat dung, 16.IV.2012, R.F.R. Melo s.n. (URM86710).

DISTRIBUTION—Asia (Japan), North America (Canada). This is the first record for the Neotropics.

COMMENTS—*Chaetomium citrinum* presents a striking diagnostic feature of greenish-yellow perithecia, especially when occurring in groups. Ascospore shapes are quite variable in this species, even irregular or with protuberances. It is a rarely encountered species of *Chaetomium*. The present material has larger perithecia than the Japanese strain (160–250 µm), as described by von Arx & al. (1986).

Chaetomium cochliodes Palliser, N.Amer. Fl. 3(1): 61 (1910) FIG. 2I–K

PERITHECIA scattered to isolated, superficial, subglobose to doliform, grayish in stereomicroscopy, dark brown to black when mounted, 310–340

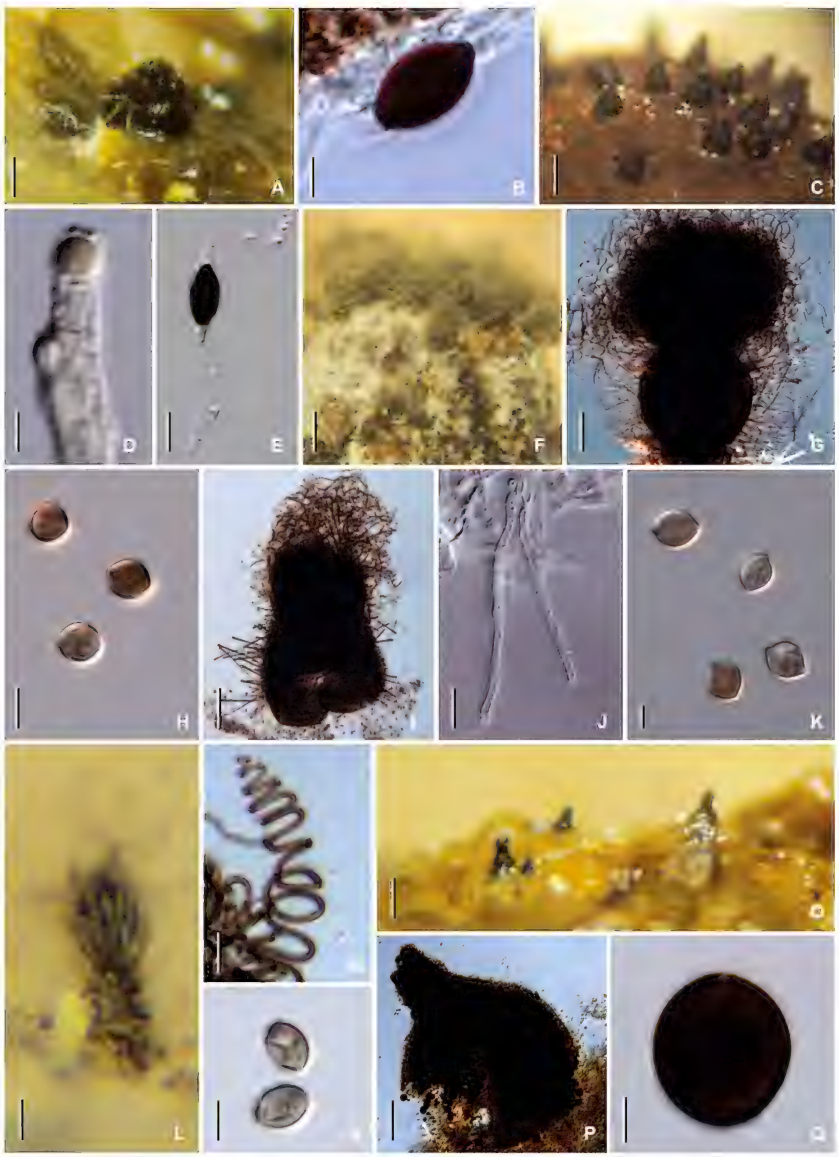
× 280–295 µm, attached to the substrate by hyaline, thin-walled rhizoids, 1.5–2.5 µm diam.; Terminal hairs of two types — (1) initially straight, from the proximal end until up to half of its length, becoming loosely spaced and coiled toward the apex, making 3–4 convolutions and progressively smaller in diameter ("corkscrew" type), brown to golden, becoming less pigmented close to the apex, extending up to 300 µm above the sporiferous head, 5–7.5 µm diam., smooth to roughened, without conspicuous septa, loosely clustered to form a weakly compact sporiferous head above the ascoma; (2) wavy, thinner, flexuous, septate, not forming coils, projecting above the sporiferous head, usually intertwined with the coiled hairs, markedly roughened, olivaceous brown to pale brown, up to 3 µm diam.; Lateral hairs numerous, long, filiform, tortuous, smooth, septate, thin-walled, light brown to golden, slightly less pigmented than the terminal hairs, 3.5–5.5 µm, extending ≤200 µm, slightly bulbous at the base; Peridium pseudo-parenchymatous, membranaceous, fragile, with flattened, angular to slightly elongate, slightly thick-walled cells, 7.5–15 µm diam.; Asci 8-spored, clavate, spore-bearing part 30–40 × 15–22.5 µm, with a short stipe, evanescent; Ascospores limoniform, biapiculate, light brown to olivaceous brown when mature, smooth, 8.5–10 × 7–8 µm.

Specimens examined—**BRAZIL. Pernambuco: Serra Talhada,** Instituto Agronômico de Pernambuco (IPA), on goat dung, 16.V.2012, R.F.R. Melo s.n. (URM86711a, b, c).

Distribution—Africa (South Africa), Asia (India, Japan, Nepal), Europe (Belgium, England, Netherlands, Norway), North America (Canada, USA), and South America (Argentina). This is the first record for Brazil.

Comments—*Chaetomium cochliodes* is a common species in indoor air samples, occasionally reported in herbivore dung. The material examined in Brazil was consistent with the protologue description: terminal hairs of two types: one long, simple, becoming coiled above the sporiferous head, and the other type septate, wavy, usually rough-walled. It can be confused with *C. globosum* Kunze, a common species with wide circumscription, but differing by the thinner terminal hairs (3.5–4.5 µm) and larger ascospores (9–12 × 7.5–8 µm) (Ames 1961).

Figure 2. Brazilian dung fungi: ***Cercophora anisura***. A. perithecium on dung; B. ascospore. ***Cercophora sordarioides***. C. perithecia on dung; D. ascal apical globule; E. mature ascospore. ***Chaetomium citrinum***. F. perithecia on dung; G. mounted perithecium; H. ascospores. ***Chaetomium cochliodes***. I. mounted perithecium; J. perithecial rhizoids; K. ascospores.



Chaetomium spirale. L. perithecium on dung; M. coiled terminal hair; N. ascospores. ***Sordaria lappae***. O. perithecia on dung; P. mounted perithecium; Q. ascospore. Scale bars: A, F = 250 µm; B = 7.5 µm; C = 750 µm; D, K, Q = 5 m; E, M = 15 µm; G = 100 µm; H, J, N = 10 µm; I = 150 µm; L, P = 50 µm; O = 300 µm.

Chaetomium spirale Zopf,Nova Acta Acad. Caes. Leop.-Carol. German. Nat. Cur. 42(5): 275 (1881) FIG. 2L–N

PERITHECIA scattered to isolated, superficial, globose to ovoid, grayish in stereomicroscopy, dark brown when mounted, 150–175 × 150–165 µm, attached to the substrate by weakly pigmented, light brown to brown, thin-walled rhizoids, 2.5–3 µm diam.; TERMINAL HAIRS of one type—initially curved from the proximal end until up to half of its length, becoming strongly coiled toward the apex, making 7–12 (usually nine) convolutions, ≤30 µm diam., progressively smaller in diameter towards the apex ("corkscrew" type), olivaceous brown to golden, usually not becoming less pigmented close to the apex, extending up to 350 µm above the sporiferous head, 2.5–5 µm diam., finely punctate to verrucose, loosely clustered to form a weakly compact sporiferous head above the perithecium; LATERAL HAIRS scarce, filiform, tortuous, smooth to slightly punctate, usually setose, light brown to golden, slightly less pigmented than the terminal hairs, 3.5–4 µm, extending up to 150 µm, slightly bulbous at the base; PERIDIUM pseudo-parenchymatous, membranaceous, fragile, with flattened, angular, slightly thick-walled cells, 4.5–12 µm diam.; ASCI 8-spored, clavate, spore-bearing part 32.5–37 × 7.5–12.5 µm, with a short stipe, evanescent; ASCOSPORES subglobose to limoniform, weakly biapiculate, light brown to olivaceous brown when mature, smooth, 8.5–9 × 5.5–7.5 µm.

SPECIMEN EXAMINED—**BRAZIL. PERNAMBUCO: Caruarru,** Instituto Agronômico de Pernambuco (IPA), Caruaru, on goat dung, 7.X.2011, R.F.R. Melo s.n. (URM86716).

DISTRIBUTION—Africa (Egypt, Iraq, Kenya, Kuwait, Libya, Swaziland, Sudan, Tanzania, Zambia), Asia (India, Japan, Malaysia), Europe (Czech Republic, England), North America (USA) and Oceania (Australia, New Zealand, Papua New Guinea). Probably worldwide. This is the first record for the Neotropics.

COMMENTS—Among similar *Chaetomium* species that present coiled "corkscrew" terminal hairs with convolutions progressively decreasing in diameter toward the apex, *C. spirale* differs from *C. convolutum* Chivers by its smaller, more globose perithecia and by the terminal hairs with more convolutions (7–12). It also differs from *C. cochliodes* by lacking a second type of terminal hair besides the coiled ones, and from *C. aterrimum* Ellis & Everh. ex Palliser in which the terminal hairs are uniform in diameter. The material presented here fits well the description provided by Ames (1961).

Sordariomycetes, Sordariales, Sordariaceae

Sordaria lappae Potebnia, Ann. Mycol. 5: 13 (1907) Fig. 20–q

Perithecia usually scattered, rarely isolated, semi-immersed to superficial, obpyriform to slightly obovoid, brown to golden, 475–550 × 300–315 µm, translucent, glabrous; neck cylindrical to papilliform, opaque, carbonaceous, dark brown to finally black close to the apex, 150–170 × 140–150 µm; Peridium pseudo-parenchymatous, membranaceous, subopaque, darkening towards the neck to finally black—outer peridial layer with angular cells, thick-walled, 7.5–17.5 µm long, occasionally inflated; Paraphyses not observed; Asci 8-spored, cylindrical, 160–190 × 16–17.5 µm, with a short stipe and tapered apex; Ascospores obovoid to subglobose, light brown to golden when young, dark brown when mature, smooth, 18–22.5 × 15–17.5 µm, guttulate, with a basal germ pore, surrounded by a thin, hyaline gelatinous sheath except at the germ pore.

Specimens examined—**BRAZIL. Pernambuco: Recife,** Universidade Federal Rural de Pernambuco (UFRPE), on horse dung, 04.IV.2012, R.F.R. Melo s.n. (URM86770a, b).

Distribution—Africa (Kenya, Tanzania, Uganda, Zaire), Europe (Czech Republic, England, Finland, France, Italy, Russia, Sweden), North America (Canada, USA) and South America (Argentina, Colombia). Possibly worldwide. This is the first record for Brazil.

Comments—*Sordaria lappae*, although recorded in many countries, is of difficult determination. *Sordaria fimicola* (Roberge ex Desm.) Ces. & De Not. differs from *S. lappae* by its less brightly coloured peridium and less inflated cells and by its smaller and less apically rounded ascospores (15–22.5 × 10–12.5 µm) (Lundqvist 1972). The material from Brazil presents slightly wider ascospores when compared to the material described by Lundqvist (13–15 µm; 1972).

Discussion

Recent surveys and inventories examining understudied substrates in tropical areas has led to novel information on the geographical distribution of fungal species. Coprophilous fungi as an ecological group are considered to occur worldwide, but their occurrence is closely tied to herbivore occurrence, so the factors that determine their geographical range are still subject to discussion. Considering that the discovery of new fungal records is quite frequent, this group may serve as an indicator of the advances in taxonomical and ecological knowledge in a region. In the present

study, representatives of three major ascomycete groups were recorded. *Sordariomycetes* represents a large clade, including non-lichenized ascomycetes with perithecial ascomata (Zhang & al. 2006). *Arnium hirtum* is known from Africa, Europe, and North America. The new record for South America suggests that this species may have a worldwide distribution. Members of *Chaetomium*, recently studied by Wang & al. (2016), were reported for the first time in South America. Although not specialized on dung, these fungi can be common in cellulose-rich substrates, and incubation in moist chambers could subvert limiting factors that would otherwise prevent its sampling and identification. *Leotiomycetes* contains mostly forms formerly known as inoperculate discomycetes: apothecial ascomycetes with asci with a non-operculum apical apparatus (Wang & al. 2006). *Thelebolales* is the main group of this class that occurs on herbivore dung. The discovery of *Coprotus albidus* updates the *Coprotus* species from Brazil recently studied by Melo & al. (2015a). Among the *Pezizomycetes*, usually regarded as "operculate discomycetes," *Ascobolaceae* species are the most well known on herbivore dung. The *Ascobolus castaneus* record is the most significant for the group, considering that *A. castaneus* was previously recorded only from China. Similarly, *Syncephalis* Tiegh. & G. Le Monn., a zygosporic mycoparasite of coprophilous ascomycetes previously recorded only in Taiwan, produced in Brazil two second worldwide records: *S. clavata* (Melo & al. 2011) and *S. obliqua* (Melo & al. 2015b). The coprophilous mycobiota of Brazil is obviously rich and diverse, and further studies will increase our understanding of their patterns of occurrence, distribution, and substrate not only in South America but on other continents as well.

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Literature cited

Ames LM. 1961. A monograph of the *Chaetomiaceae*. U.S. Army, Research and Development, Washington DC. 125 p.

Asgari B, Zare R, Gams W. 2007. *Coniochaeta ershadii*, a new species from Iran, and a key to well-documented *Coniochaeta* species. Nova Hedwigia 84: 175–187. https://doi.org/10.1127/0029-5035/2007/0084-0175

Basumatary SK, McDonald HG. 2017. Coprophilous fungi from dung of the greater one-horned rhino in Kaziranga National Park, India and its implication to paleoherbivory and paleoecology. Quaternary Research 88: 14–22. https://doi.org/10.1017/qua.2017.34

Bell A. 1983. Dung fungi: an illustrated guide to coprophilous fungi in New Zealand. Victoria University Press, Wellington. 88 p.

Bell A. 2005. An illustrated guide to the coprophilous *Ascomycetes* of Australia. CBS Biodiversity Series 3. 172 p.

Brummelen J van. 1967. A world-monograph of the genera *Ascobolus* and *Saccobolus* (*Ascomycetes*, *Pezizales*). Persoonia Supplement 1. 260 p.

Calaça FJS, Silva NC da, Xavier-Santos S. 2014. A checklist of coprophilous fungi and other fungi recorded on dung from Brazil. Mycotaxon mycobiota [22 p.] http://www.mycotaxon.com/resources/checklists/calaca_v128_checklist.pdf [Abstract: Mycotaxon 128: 205. https://doi.org/10.524 8/128.205].

Doveri F. 2004. Fungi fimicoli Italici. Associazione Micologica Bresadola, Trento. 1104 p.

Hoog GS de, Göttlig E, Platas G, Genilloud O, Leotta G, Brummelen J van. 2005. Evolution, taxonomy and ecology of the genus *Thelebolus* in Antarctica. Studies in Mycology 51: 33–76.

Kimbrough JW, Luck-Allen ER, Cain RF. 1972. North American species of *Coprotus* (*Thelebolaceae*: *Pezizales*). Canadian Journal of Botany 50: 957–971. https://doi.org/10.1139/b72-116

Kirschner R, Hsu T, Tuan NN, Chen CL, Huang SL. 2015. Characterization of fungal and bacterial components in gut/fecal microbiome. Current Drug Metabolism 16(4): 272–283. https://doi: 10.2174/1389200216666150812124625

Luck-Allen ER, Cain RF. 1975. Additions to the genus *Delitschia*. Canadian Journal of Botany 53(17): 1827–1887.

Lundqvist N. 1972. Nordic *Sordariaceae* s.lat. Symbolae Botanicae Upsalienses 20(1). 374 p.

Melo RFR, Santiago ALCMA, Cavalcanti MAQ. 2011. *Syncephalis clavata* (*Zoopagales*, *Zygomycetes*), a first record from the Neotropics. Mycotaxon 116: 113–136. https://doi.org/10.5248/116.133

Melo RFR, Bezerra JL, Cavalcanti MAQ. 2012. Diversity of coprophilous ascomycetes from captive wild animals in Dois Irmãos State Park, Brazil. Nova Hedwigia 94: 153–162. https://doi.org/10.1127/0029-5035/2012/0094-0153

Melo RFR, Miller AN, Santiago ALCMA, Maia LC. 2014. The genera *Ascobolus* and *Saccobolus* (*Ascobolaceae*, *Pezizales*) in Brazil. Mycosphere 4: 363–454. https://doi.org/10.5943/mycosphere/5/6/9

Melo RFR, Miller AN, Maia LC. 2015a. *Coprotus* (*Thelebolaceae*, *Thelebolales*) in herbivore dung from Brazil. Nova Hedwigia 101: 35–48. https://doi.org/10.1127/nova_hedwigia/2014/0232

Melo RFR, Maia LC, Santiago ALCMA. 2015b. The discovery of *Syncephalis obliqua* (*Zoopagomycotina*, *Zoopagales*) in the Neotropics. Mycotaxon 130: 1165–1169. https://doi.org/10.5248/130.1165

Melo RFR, Maia LC, Miller AN. 2017. Coprophilous ascomycetes with passive ascospore liberation from Brazil. Phytotaxa 295: 159–172. https://doi.org/10.11646/phytotaxa.295.2.4

Newcombe G, Campbell J, Griffith D, Baynes M, Launchbaugh K, Pendleton R. 2016. Revisiting the life cycle of dung fungi, including *Sordaria fimicola*. PLoS ONE 11(2): e0147425 [11 p.]. https://doi.org/10.1371/journal.pone.0147425

Richardson MJ. 2001. Diversity and occurrence of coprophilous fungi. Mycological Research 105: 387–402. https://doi.org/10.1017/s0953756201003884.

Richardson MJ, Watling R. 1997. Keys to fungi on dung, 2nd ed. Stourbridge, British Mycological Society. 68 p.

Souza CAF de, Lima DC, Gurgel LMS, Santiago ALCMA. 2017. Coprophilous *Mucorales* (ex *Zygomycota*) from three areas in the semi-arid of Pernambuco, Brazil. Brazilian Journal of Microbiology 48: 79–86. https://doi.org/10.1016/j.bjm.2016.09.008

von Arx JA, Guarro J, Figueras MJ. 1986. The ascomycete genus *Chaetomium*. Beihefte zur Nova Hedwigia 84. 162 p.

Wang Z, Johnston PR, Takamatsu S, Spatafora JW, Hibbett DS. 2006. Toward a phylogenetic classification of the *Leotiomycetes* based on rDNA data. Mycologia 98: 1065–1075. https://doi.org/10.1080/15572536.2006.11832634

Wang XW, Houbraken J, Groenewald JZ, Meijer M, Andersen B, Nielsen KF, Crous PW, Samson RA. 2016. Diversity and taxonomy of *Chaetomium* and chaetomium-like fungi from indoor environments. Studies in Mycology 84: 145–224. https://doi.org/10.1016/j.simyco.2016.11.005

Zhang N, Castlebury LA, Miller AN, Huhndorf SM, Schoch CL, Seifert KA, Rossman AY, Rogers JD, Kohlmeyer J, Volkmann-Kohlmeyer B, Sung GH. 2006. An overview of the systematics of the *Sordariomycetes* based on a four-gene phylogeny. Mycologia 98: 1076–1087. https://doi.org/10.3852/mycologia.98.6.1076

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Two *Heterostelium* species newly recorded from China

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Abstract—Two species of dictyostelid cellular slime molds new to China (*Heterostelium anisocaule* and *H. arachnoideum*) were isolated from samples of soil/humus collected in East China. Descriptions and illustrations based on these isolates are provided.

Key words—*Amoebozoa,* taxonomy, forest soils

Introduction

Dictyostelid cellular slime molds (dictyostelids) are key components of the soil microbiota and play a role in maintaining the balance that exists between bacteria and other organisms (Raper 1984). Dictyostelids have been reported from several localities in China (He & Li 2010; Liu & Li 2012a,b, 2014, 2017; Liu & al. 2019), but there are relatively few reports of this group from East China (Yeh & Chien 1983, Hagiwara & al. 1985, Zhao & al. 2017). East China includes Shandong Province, Jiangsu Province, Anhui Province, Zhejiang Province, Jiangxi Province, Fujian Province, Shanghai, and Taiwan. Two species of dictyostelids, reported originally from the Southern Hemisphere, were isolated from samples of soil/humus collected from this region. Descriptions and illustrations based on these isolates are provided herein.

Materials & methods

Samples were collected during 2012 from Jiangsu Province, Fujian Province, and Taiwan. Each sample consisted of 30–50 g of soil/humus and was placed in a sterile whirl-pack plastic bag. Each sample bag was numbered and the sample itself preserved

at 4 °C in the herbarium of the Mycological Institute of Jilin Agricultural University, Changchun, China (HMJAU). Isolation methods followed Cavender & Raper (1965), with some minor modifications outlined by Liu & al. (2019). Isolates were identified with reference to Raper (1984) and following the new classification system of Sheikh & al. (2018). The characteristic stages in the life cycle, including cell aggregation and the formation of pseudoplasmodia and sorocarps, were observed under a Zeiss Axio Zoom V16 dissecting microscope with a 1.5× objective and 10× ocular. Slides with sorocarps were prepared with water as the mounting medium. Features of spores, sorophores, and sorocarps were observed and measured using a Zeiss Axio Imager A2 light microscope with 10× ocular and 10, 40, and 100× (oil) objectives. Photographs were taken with Zeiss Axiocam 506 color microscope camera. Spores from these plates were frozen in HL 5 media (Cocucci and Sussman 1970) and stored at − 80 °C in HMJAU.

Taxonomy

Heterostelium anisocaule (Cavender & al.) S. Baldauf, S. Sheikh & Thulin, Protist 169: 14. 2018. PLATE 1a–e

When cultured at 23 °C on non-nutrient agar with *Escherichia coli*, sorocarps white, erect to prone, solitary to clustered, prostrate, phototropic, normally 3–9 mm. Sorophores asymmetrical, sinuous, with 1–7 whorls, each whorl comprising 1–4 branches. Sorophore tips and the upper portion of the sorophore consist of one tier of cells, bases round. Terminal sori white, globose, commonly 60–160 µm in diam. Lateral sori white, globose, 15–85 µm in diam. Spores elliptical 5–9.5×3–5.5 µm, with unconsolidated granules. Aggregations of the "violaceum" type.

SPECIMEN EXAMINED– **CHINA**, **JIANGSU PROVINCE**, **Jintan**, Mao Mountains, soil (S2593) collected in broadleaf forest, 19 Aug. 2012, Liu & al.; isolated in 2014 (HMJAU MR174).

COMMENTS—*Heterostelium anisocaule* was first isolated from a sample collected in a podocarp/broadleaf forest in Northland, New Zealand. This species has a wide range of temperature adaptation (18–30 °C). Variations in temperature and light exposure result in a considerable variation in sorocarp size. The optimum temperature is 22–23 °C (Cavender & al. 2002).

Heterostelium arachnoideum (Vadell & Cavender) S. Baldauf, S. Sheikh & Thulin, Protist 169: 14. 2018. PLATE 1f–n

When cultured at 23 °C on non-nutrient agar with *E. coli*, sorocarps white, erect to prone, sometimes prostrate, solitary to clustered, with slightly phototropic growth, 5–14 mm or more. Sorophores asymmetrical, with 3–20 whorls, each whorl with 2–5 uneven branches. The lower portion of the

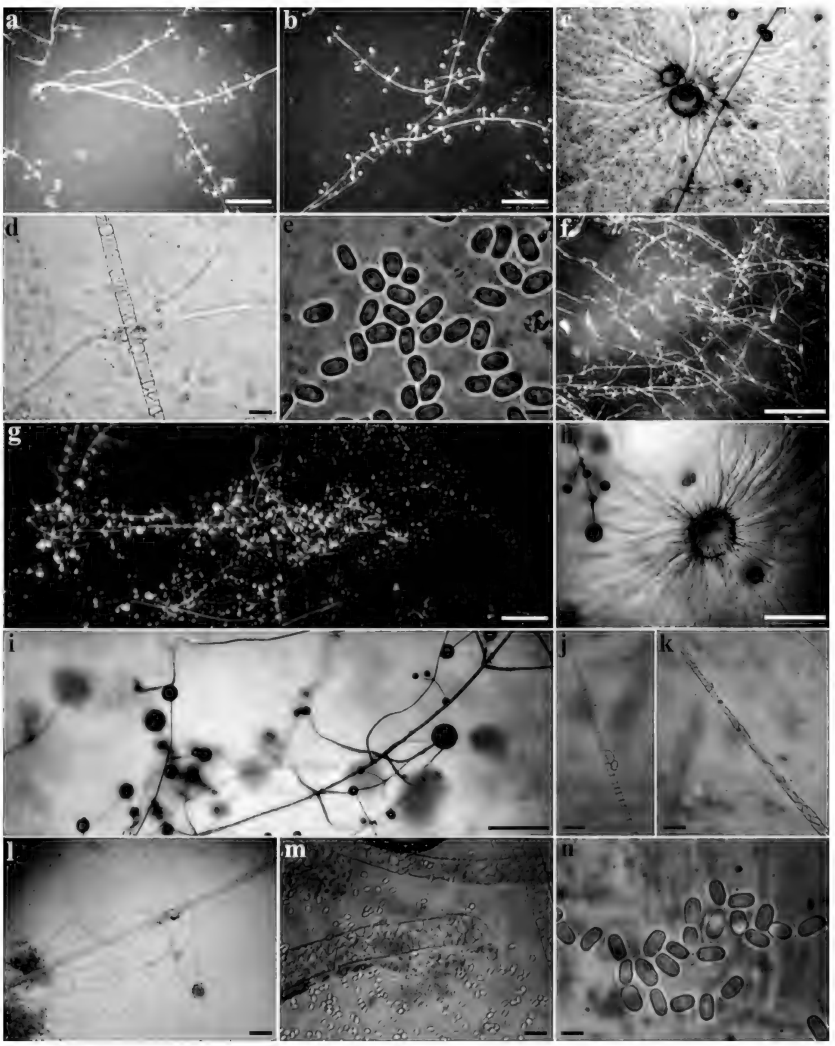

Plate 1. ***Heterostelium anisocaule***: a, b. Sorocarps; c. Aggregation; d. Sorophores and branches; e. Spores. ***Heterostelium arachnoideum***: f, g, i. Sorocarps; h. Aggregation; j, k. Sorophore tip; l. Sorophores and branches; m. Sorophore base; n. Spores. Scale bars: a, b, f, g = 1 mm; c,h = 100 μm; d, j, k = 20 μm; e, n = 5 μm; i = 0.5 mm; l = 50 μm; m = 30 μm.

sorophore sometimes with secondary whorls. Sorophore tips consist of one tier of cells, sinuous, becoming tangled together to form a delicate spider-web-like structure, especially sometimes without sori present. Bases clavate,

irregular. Terminal sori white, globose, commonly 30–135 µm in diam. Lateral sori white, globose, commonly 20–50 µm in diam. Spores elliptical or oblong, mostly 5.5–8×3–4.5, with unconsolidated polar granules. Aggregations of the "violaceum" type. Late sorogens sometimes curved.

Specimen examined– Specimen examined: **CHINA, Jiangsu Province, Yixing**, soil (S2570) collected in broadleaf forest, 22 Aug. 2012, Liu & al.; isolated in 2014 (HMJAU MR175). **Fujian Province, Fuzhou**, Gu Mountain, soil (S2031) collected in mixed forest, 11 Mar. 2012, Liu & al.; isolated in 2014 (HMJAU MR209). **Taiwan, Yilan**, Lotung Sports Park, soil (S2936) collected in humus, 11 Sep. 2012, Liu & al.; isolated in 2014 (HMJAU MR210).

Comments—*Heterostelium arachnoideum* was originally isolated from soil litter collected from three sites within Iguazú National Park, Misiones, Argentina. This species is characterized by its spider web-like terminal sorophores (Vadell & Cavender 2007).

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Literature cited

Cavender JC, Raper KB. 1965. The *Acrasiae* in nature. I. Isolation. American Journal of Botany 52: 294–296.

Cavender JC, Stephenson SL, Landolt JC, Vadell EM. 2002. Dictyostelid cellular slime moulds in the forests of New Zealand. New Zealand Journal of Botany 40: 235–264. https://doi.org/10.1080/0028825X.2002.9512786

Cocucci, S.M., Sussman, M., 1970. RNA in cytoplasmic and nuclear fractions of cellular slime mold amebas. Journal of Cell Biology 45: 399–407. https://doi.org/10.1083/jcb.45.2.399

Hagiwara H, Yeh ZY, Chien CY. 1985. *Dictyostelium macrocephalum*, a new dictyostelid cellular slime mold from Taiwan. Bulletin of the National Science Museum, Series B, 11: 103–108.

He XL, Li Y. 2010. A new species of *Dictyostelium* from Tibet, China. Mycotaxon 111: 287–290. https://doi.org/10.5248/111.287

Liu P, Li Y. 2012a. Dictyostelids from Heilongjiang Province, China. Nova Hedwigia 94(1–2): 265–270. https://doi.org/10.1127/0029-5035/2012/0094-0265

Liu P, Li Y. 2012b. New records of dictyostelids from China. Nova Hedwigia 94(3–4): 429–436. https://doi.org/10.1127/0029-5035/2012/0010

Liu P, Li Y. 2014. Dictyostelids from Jilin Province, China. I. Phytotaxa 183(4): 279–283. https://doi.org/10.11646/phytotaxa.183.4.7

Liu P, Li Y. 2017. Dictyostelids from Jilin Province, China. II. Phytotaxa 323(1): 077–082. https://doi.org/10.11646/phytotaxa.323.1.6

Liu P, Zou Y, Li Shu, Stephenson SL, Li Y. 2019. Two new species of dictyostelid cellular slime molds in high-elevation habitats on the Qinghai-Tibet Plateau, China. Scientific Reports 9:5 [13 p.]. https://doi.org/:10.1038/s41598-018-37896-7

Raper KB. 1984. The Dictyostelids. Princeton University Press, Princeton, New Jersey.

Sheikh S, Thulin M, Cavender JC, Escalante R, Kawakami S, Lado C, Landolt JC, Nanjundiah V, Queller DC, Strassmann JE, Spiegel FW, Stephenson SL, Vadell EW, Baldauf SL. 2018. A new classification of the dictyostelids. Protist 169: 1–28. https://doi.org/10.1016/j.protis.2017.11.001.

Vadell EM, Cavender JC. 2007. Dictyostelids living in the soils of the Atlantic Forest, Iguazú region, Misiones, Argentina: description of new species. Mycologia 99(1): 112–124. https://doi.org/10.1080/15572536.2007.11832606

Yeh ZY, Chien CY. 1983. Cellular slime molds in Taiwan I: Four newly recorded species. Biological Bulletin of National Taiwan Normal University 18: 69–86.

Zhao M, Liu P, An Y, Yao Y, Li Y. 2017. *Dictyostelium annularibasimum* (*Dictyosteliaceae*, *Dictyostelida*), a new purple species from China. Nova Hedwigia 104(1–3): 351–358. https://doi.org/info:doi/10.1127/nova_hedwigia/2016/0381

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Pseudocercospora meliosmicola sp. nov. and three new *Pseudocercospora* records from China

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Abstract—A new species, *Pseudocercospora meliosmicola*, and three new records, *P. deglupta*, *P. eucalyptigena*, and *P. infuscans*, are reported from China. Descriptions, illustrations, and discussions of the species concerned are provided. The examined specimens are deposited in HMAS.

Key words—biodiversity, cercosporoid fungi, *Meliosma parviflora*, *Mycosphaerellaceae*, taxonomic novelty

Introduction

Pseudocercospora Speg. (Spegazzini 1910), with more than 1600 names recorded, represents the largest genus of cercosporoid hyphomycetes. The genus comprises plant pathogens either causing distinct necrotic spots or forming effuse fungal colonies without any definite spots, on leaves, stems, flowers, and fruits. Some species are known to cause severe diseases on important crops, which is also reflected in plant quarantine regulations. On the other hand, some have been used as biological control agents of weeds (Den Breeÿen & al. 2006). More than 400 *Pseudocercospora* species have been reported from China (Liu & Guo 1998). Here we propose *Pseudocercospora meliosmicola* on *Meliosma parviflora* as a new species, and report *P. deglupta*, *P. eucalyptigena*, and *P. infuscans* as new records for China.

Taxonomy

Pseudocercospora deglupta Crous, Mycol. Mem. 21: 127, 1998. FIG. 1

Leaf spots amphigenous, subcircular to angular, without definite margin, 1–5 mm wide, confluent, pale gray to gray to brown, with a pale gray to pale olivaceous brown halo on the upper surface, pale grayish brown to pale brown on the lower surface. Fruiting hypogenous. Mycelium internal. Stromata absent or well-developed, substomatal, subglobose, dark brown, 20–40 µm diam. Conidiophores few, emerging through stomata, or in dense fasciculate, olivaceous brown to dark olivaceous brown, paler towards the apex, regular in width, cylindrical, unbranched or branched, smooth

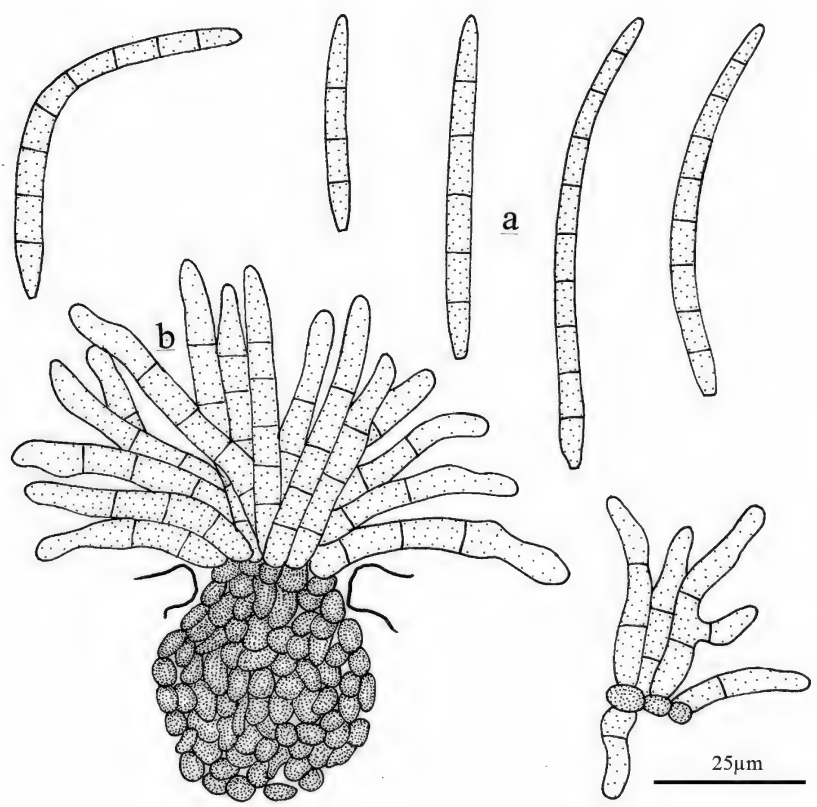

FIG. 1 *Pseudocercospora deglupta* (HMAS 247119).
a. Conidia; b. Conidiophores. Scale bar = 25 µm.

to finely verruculose, straight to curved, obtuse to conical at the apex, 1–7-septate, 20–60(–80) × 3.2–5.4 μm. Conidiogenous loci inconspicuous, unthickened and not darkened. Conidia solitary, obclavate-cylindrical to cylindrical, olivaceous to moderately olivaceous brown, straight to cured, finely verruculose, obtuse at the apex, obconically truncate at the base, hila neither thickened not darkened, 3–10-septate, 32–90 × 3–4.5 μm.

Specimen examined: On living leaves of *Eucalyptus globulus* Labill. (*Myrtaceae*): **China**, **Yunnan Province**, Fenglu, 27.IX.1987, coll. Guo Y.L. no. 1141 (HMAS 247119).

Notes: *Pseudocercospora basitruncata* Crous on *Eucalyptus* sp. is similar to *P. deglupta*, but differs in having amphigenous fruiting, sympodially or percurrently proliferating conidiogenous cells, and paler (hyaline to olivaceous) and narrower conidia (25–70(–90) × 2.5–3(–3.5) μm; Crous 1998).

Pseudocercospora eucalyptigena U. Braun, Fungal Diversity 8: 42, 2001. Fig. 2

Leaf spots amphigenous, irregularly angular, without definite margin, 1–4.5 mm wide, vein-limited, often confluent, yellowish brown to brown on the upper surface, pale brown to graying brown on the lower surface. Fruiting hypogenous. Mycelium internal and external; hyphae pale olivaceous to olivaceous, branched, smooth, septate, 2–3 μm wide. Stromata absent or small, substomatal, subglobose, olivaceous brown, 10–30 μm diam. Conidiophores few, emerging through stomatal, loose fasciculate or arising singly as lateral branches from external mycelial hyphae, pale olivaceous to pale olivaceous brown, paler towards the apex, irregular in width, not branched, straight to curved, 0–1-geniculate, denticulate, obtuse to conical at the apex, 0–1(–2)-septate, primary conidiophores 8–35 × 2.5–3.5 μm, secondary conidiophores (arising from superficial hyphae) 5–25 × 2.5–3.5 μm. Conidiogenous loci inconspicuous, unthickened and not darkened. Conidia solitary, cylindrical to cylindrical-obclavate, pale olivaceous, straight to strongly curved, acute to obtuse at the apex, subtruncate to obconically truncate at the base, hila neither thickened nor darkened, indistinctly 3–12-septate, 35–80(–100) × 2–3 μm.

Specimen examined: On living leaves of *Eucalyptus* sp. (*Myrtaceae*): **China**, **Taiwan**, Taibei, 20.VII.1926, coll. Sawada K., no. 9196 (HMAS 05196).

Notes: *Pseudocercospora cubae* Crous, *P. deglupta* Crous, and *P. denticulata* Crous on *Eucalyptus* sp. all differ from *P. eucalypticola* in having finely verruculose conidiophores and conidia. Additionally, *P. cubae* differs in having

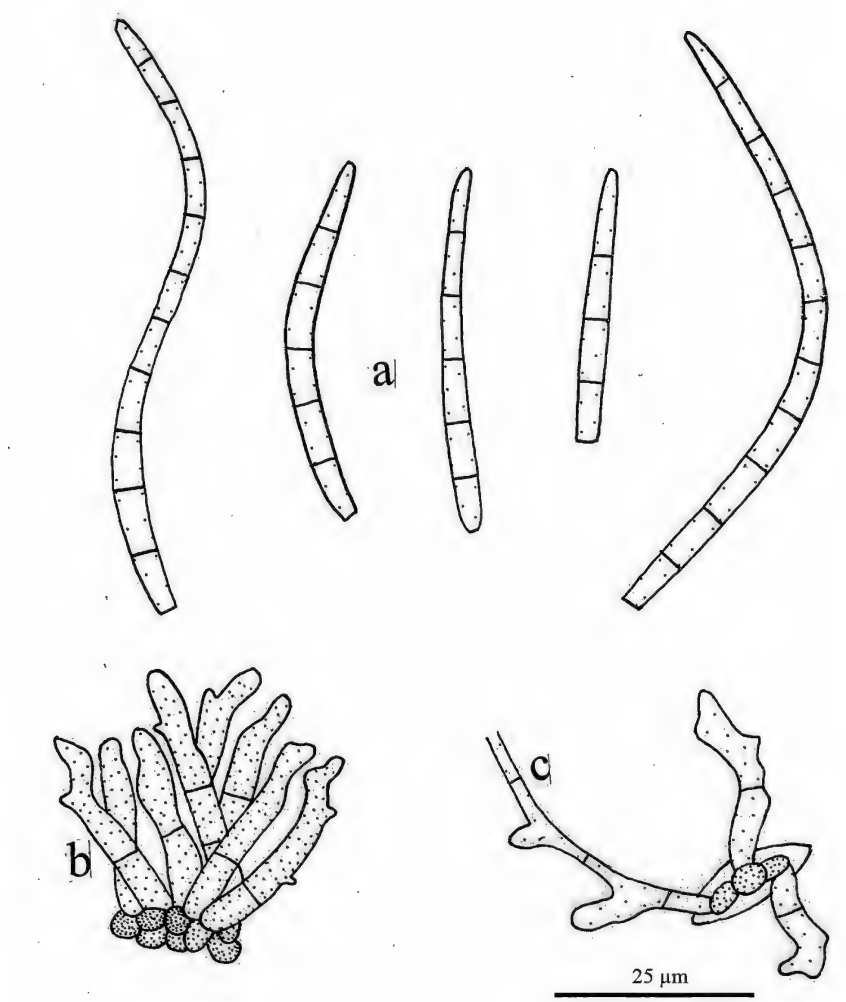

Fig. 2 *Pseudocercospora eucalyptigena* (HMAS 05196).
a. Conidia; b. Conidiophores; c. Stroma. Scale bar = 25 µm.

sympodially or percurrently proliferating conidiogenous cells (Crous 1998); *P. deglupta* possesses longer and wider conidiophores (60–120 × 4–6 µm) and wider conidia (3.0–4.0(–4.5 µm); Crous 1998); and *P. denticulata* has amphigenous fruiting, larger stromata (60 µm wide, 30 µm high), and darker (moderately brown), wider conidiophores (3–5 µm; Crous 1998).

Pseudocercospora infuscans (Ellis & Everh.) U. Braun,
Monogr. Cercosp. Ramul. Allied Gen. 2: 402, 1998. FIG. 3

MISAPPLIED: *Pseudocercospora toxicodendri* sensu Liu & Guo (1989)

Leaf spots amphigenous, subcircular to angular, 1–4 mm wide, grayish white to red-brown on the upper surface, yellowish brown to pale red-brown on the lower surface. Fruiting hypogenous. Mycelium internal and

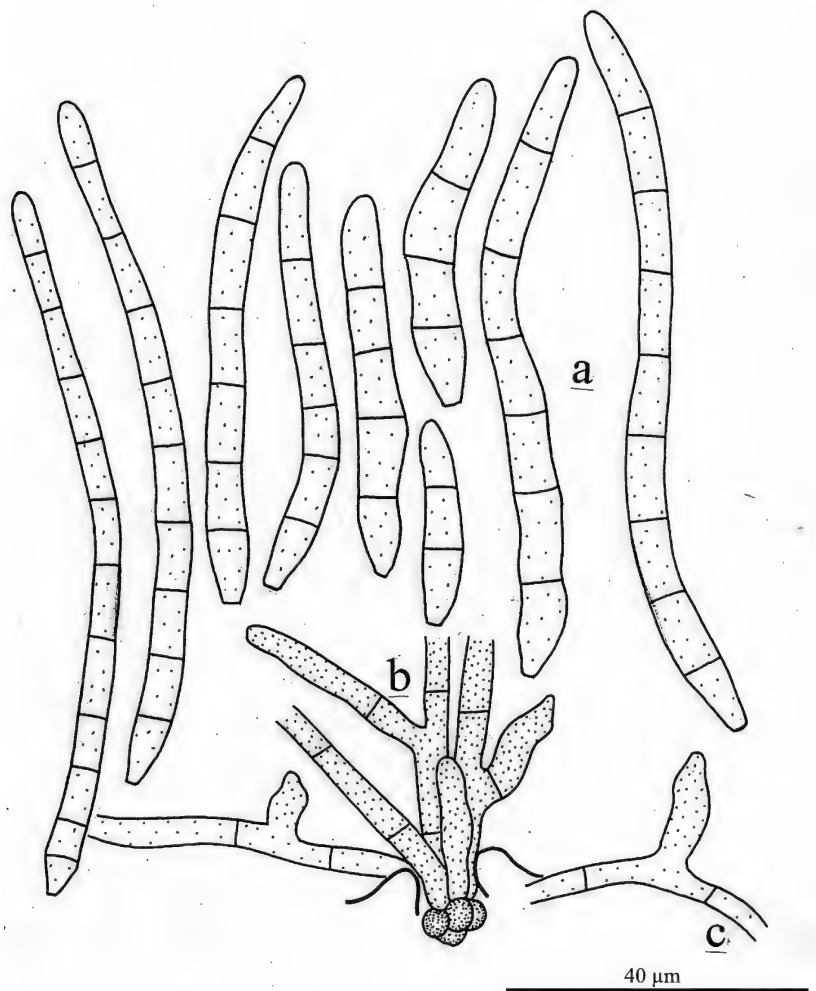

FIG. 3 *Pseudocercospora infuscans* (HMAS 60028).
a. Conidia; b. Conidiophores; c. Stroma. Scale bar = 40 μm.

external, subhyaline, branched, smooth, septate, 2–3 µm diam. Stromata absent. Conidiophores 2–5, emerging through stomata or arising singly as lateral branches from external mycelial hyphae, olivaceous to pale olivaceous brown, uniform in color, irregular in width, branched, geniculate, denticulate, straight to curved, rounded to conical at the apex, 1–7-septate, sometimes constricted, 6.5–125 × 3–5.5 µm. Conidiogenous loci inconspicuous, unthickened and not darkened. Conidia solitary, cylindrical to cylindrical-obclavate, pale olivaceous, straight to curved, obtuse at the apex, obconically truncate at the base, hila neither thickened not darkened, 2–11-septate, 30–120 × 3–6.5 µm.

Specimens examined: On living leaves of *Rhus chinensis* Mill. (*Anacardiaceae*): **China**, **Hunan Province**, Longshan, 28.VIII.1958, coll. Chen Q.T., Liang L.S. no. 135 (HMAS 55957); no. 143 (HMAS 55958). **Sichuan Province**, Emeishan, 17.X.1958, coll. Song M.H. no. 235 (HMAS 60028).

Notes: Guo & Liu (1989) introduced the combination *Pseudocercospora toxicodendri* (Ellis) X.J. Liu & Y.L. Guo (≡ *Cercospora toxicodendri* Ellis) and erroneously applied this name to *Pseudocercospora* collections on *Rhus chinensis* Mill. in China. Based on examination of the type material, Braun (1998) revealed that *C. toxicodendri* represents a species of *Cercosporella*, reallocated *Cercospora infuscans* to *Pseudocercospora,* and emphasized that the Chinese collections, erroneously assigned to *P. toxicodendri*, agree with *P. infuscans*, although the conidia are somewhat narrower (3–5.5 µm), and the conidia longer (to 120 µm) compared to North American samples, which are characterized by broader (3–8 µm) conidiophores and shorter (30–70) conidia (Braun 1998). The re-examination of the Chinese collections on *R. chinensis* cited under "specimens examined" showed that they should be properly referred to as *Pseudocercospora infuscans* as emphasized by Braun (1998) and Braun & al. (2016).

Pseudocercospora meliosmicola Y.L. Guo, Qian Zhao & Y.X. Shi, **sp. nov.** Fig. 4

MB 823728

Differs from *Pseudocercospora sabiae* by its longer, denticulate conidiophores and its paler, longer conidia.

Type: China, Zhejiang Province, Hangzhou, on living leaves of *Meliosma parviflora* Lecomte (*Sabiaceae*), 27.IX.1961, coll. Ma Qi-ming, Liu Xi-jin 581 (**Holotype**, HMAS 168350).

Etymology: Derived from the host genus *Meliosma.*

Leaf spots amphigenous, circular to angular, 1–6 mm wide, often confluent, red-brown, dark brown to almost black, with a pale olivaceous brown halo

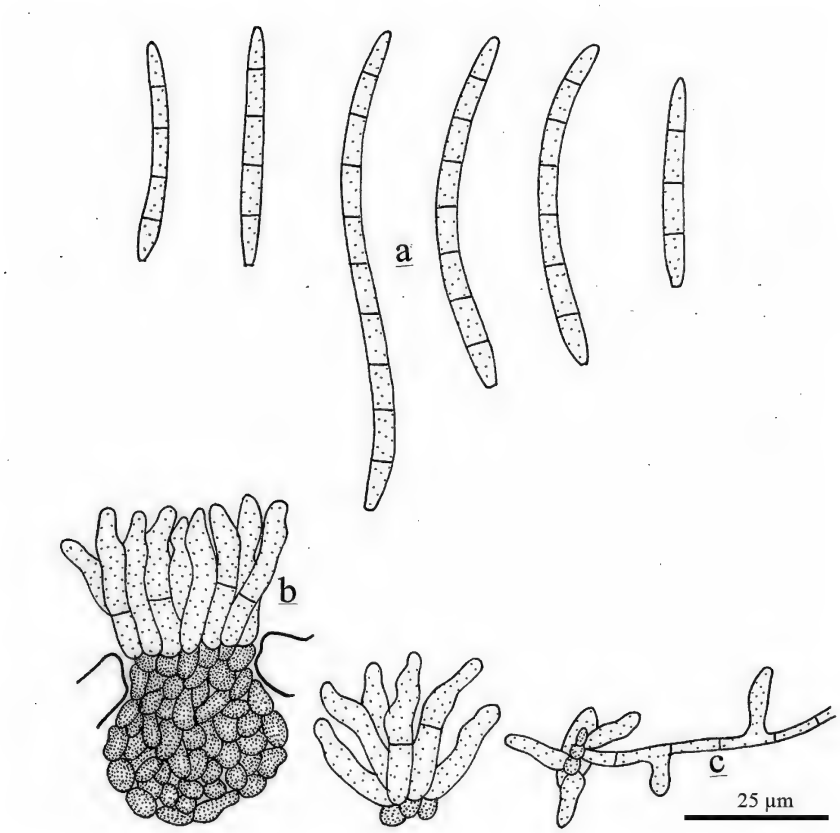

Fig. 4 *Pseudocercospora meliosmicola* (holotype, HMAS 168350).
a. Conidia; b. Conidiophores; c. Stroma. Scale bar = 25 µm.

on the upper surface, olivaceous brown to pale brown, with an olivaceous brown halo on the lower surface. Fruiting amphigenous. Mycelium internal and external; hyphae olivaceous, branched, smooth, septate, 2–2.5 µm wide. Stromata substomatal, only a few brown globose cells to well-developed, subglobose, dark brown, 20–40 µm diam. Conidiophores few, emerging through stomata, loose to dense fasciculate or arising singly as lateral branches from external mycelial hyphae, olivaceous to pale olivaceous brown, uniform in color, irregular in width, straight to geniculate-curved, branched, smooth, rounded to conical at the apex, 0–1-septate, 8–35 × 3–3.5(–4) µm. Conidiogenous cells cylindrical, 8–24

µm long, conidiogenous loci inconspicuous, unthickened, not darkened. Conidia solitary, cylindrical to narrowly obclavate-cylindrical, olivaceous to very pale olivaceous brown, smooth, straight to curved, obtuse at the apex, obconically truncate at the base, hila neither thickened nor darkened, indistinctly 3–7(–10)-septate, 25–70(–85) × 2.5–3.5 µm.

Notes: Cercosporoid fungi previously reported on hosts in *Sabiaceae* include *Pseudocercospora sabiae* Y.L. Guo & W.X. Zhao on *Sabia* sp. (Guo & Zhao 1989) and *Cercospora meliosmae* Y.L. Guo & Li Xu on *Meliosma* sp. (Guo & Xu 2004). *Pseudocercospora sabiae* is distinguished from *P. meliosmicola* by its epigenous fruiting habit; conidiophores that are darker (olivaceous to pale brown), denticulate, and longer (15–162 µm); and conidia that are obclavate to cylindrical, paler (pale olivaceous), and longer and slightly wider (40–112.5 × 3–4.3 µm; Guo & Zhao 1989). Its placement in another genus clearly separates *Cercospora meliosmae* from *Pseudocercospora meliosmicola*.

Acknowledgments

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Literature cited

Braun U. 1998. A monograph of *Cercosporella, Ramularia* and allied genera (phytopathogenic hyphomycetes). Eching: Ihw-Verlag 2: 402 p.

Braun U, Crous PW, Nakashima C. 2016. Cercosporoid fungi (*Mycosphaerellaceae*) 5. Species on dicots (*Anacardiaceae* to *Annonaceae*). IMA Fungus 7(1): 161–216. https://doi.org/10.5598/imafungus.2016.07.01.10

Crous PW. 1998. *Mycosphaerella* spp. and their anamorphs associated with leaf spot diseases of *Eucalyptus*. Mycologia Memoirs 21: 170 p.

Den Breeÿen A, Groenewald JZ, Verkley GJM, Crous PW. 2006. Morphological and molecular characterisation of *Mycosphaerellaceae* associated with the invasive weed, *Chromolaena odorata*. Fungal Diversity 23: 89–110.

Guo YL, Liu XJ. 1989. Studies on the genus *Pseudocercospora* in China I. Mycosystema 2: 225–240.

Guo YL, Xu L. 2004. A new species of *Cercospora* causing leaf spot of *Meliosma* sp. Mycosystema 23: 18–19.

Guo YL, Zhao WX. 1989. Studies on hyphomycetes of Zhangjiajie in Hunan I. *Pseudocercospora*. Acta Mycologica Sinica 8: 118–122.

Liu XJ, Guo YL. 1998. Flora fungorum sinicorum vol. 9: *Pseudocercospora*. Beijing: Science Press.

Spegazzini CL. 1910. Mycetes argentinenses (Series V). Anales del Museo Nacional de Historia Natural de Buenos Aires 20: 329–467.

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Heteroplacidium compactum reported as a genus new to China

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Abstract—Three specimens of *Heteroplacidium compactum* (*Verrucariaceae*), isolated from arid areas in Northwest China, were identified by morphological and phylogenetic comparisons with other species, including species of *Endocarpon* and *Placidium* (common verrucariaceous genera in the same area). This is the first report of *Heteroplacidium* from China.

Key words—desert, ITS, lichen, morphology, taxonomy

Introduction

Breuss (1996) separated four genera—*Anthracocarpon*, *Clavascidium*, *Heteroplacidium*, and *Involucropyrenium*—from *Catapyrenium* Flot. s. lat. (*Verrucariaceae*) based on morphological characters. *Neocatapyrenium* H. Harada, *Placidium* A. Massal., and *Scleropyrenium* H. Harada are also morphologically related (Prieto & al. 2010b) but are independent monophyletic lineages within *Verrucariaceae*, according to phylogenetic analyses (Gueidan & al. 2007, 2009; Prieto & al. 2010b).

Endocarpon Hedw. (*Verrucariaceae*) was relatively common during our field survey in the arid region of Northwest China (Yang & Wei 2008, Zhang & al. 2017a), but no species of *Heteroplacidium* Breuss was recorded. *Heteroplacidium,* which grows primarily on soil and rock in warm temperate

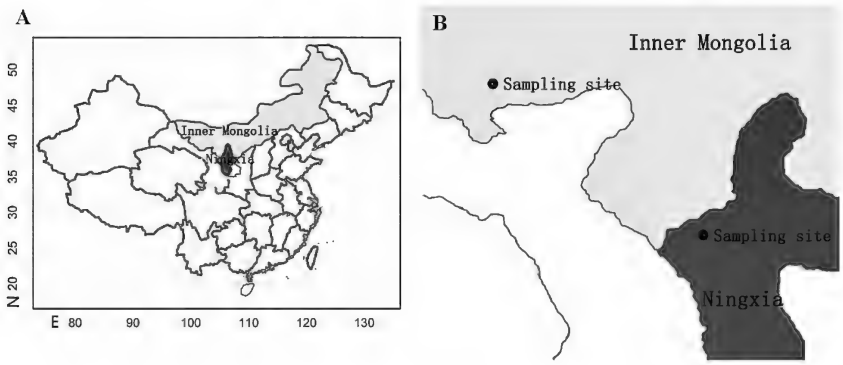

Fig. 1. *Heteroplacidium compactum* collection sites in China.

regions, is characterized by a crustose-areolate to squamulose thallus, a paraplectenchymatous or subparaplectenchymatous cortex, and clavate asci with biseriate ascospores (Prieto & al. 2010a); twelve species have been accepted (Knudsen & al. 2014). We report *Heteroplacidium* in China for the first time, based on three specimens of *H. compactum*.

Material & methods

Phenotypic analysis

All *Heteroplacidium* samples were collected from Ningxia Hui Autonomous Region and Inner Mongolia (Fig. 1) and are preserved in the Lichen Section of Herbarium Mycologicum Academiae Sinicae, Beijing, China (HMAS-L). Zeiss Stemi SV11 dissecting and Zeiss Axioskop 2 plus compound microscopes were used for morphological examinations.

DNA extraction, amplification, sequencing

DNA was extracted from our three fresh specimens of *Heteroplacidium* (Table 1) following the modified CTAB method (Rogers & Bendich 1988). The internal transcribed spacer of nuclear ribosome DNA (nrDNA ITS), the standard DNA barcoding marker (Schoch & al. 2012), was chosen as the genetic marker. Primers LR1 (Vilgalys & Hester 1990) and ITS1 (White & al. 1990) were used. Reactions were carried out in 50 µL reaction volume comprising 3 µL total DNA, 1 µL each primer (10 µM), 25 µL 2×Taq MasterMix (CWBIO) and 20 µL ddH_2O. The DNA was amplified in a Biometra T-Gradient thermal cycler as follows: initial heating for 5 min at 95 °C; 35 cycles of 30 s at 94 °C, 30 s at 56 °C, and 90 s at 72 °C; ending with a final extension of 8 min at 72 °C, after which the samples were kept at 4 °C. Negative controls were prepared for each amplification series. PCR products were purified using a CWBIO gel extraction kit following the manufacturer's instructions.

Sequence alignment and phylogenetic tree construction

PCR products from the three putative *Heteroplacidium* specimens were sequenced using ABI 3730 XL Sequencer. Additional sequences of *Endocarpon* (3 spp.), *Heteroplacidium* (4 spp.), and *Placidium* (3 spp.) were downloaded from GenBank (Table 1) with the *Endocarpon* sequences selected as outgroup. The sequences were aligned using ClustalW Multiple Alignment (Thompson & al. 1994) in BioEdit 7.2.5 (Hall 1999). The program Gblocks v0.91b (Castresana 2000, Talavera & Castresana 2007) was used to delimit and remove regions of alignment uncertainty, using options for a "less stringent" selection on the Gblocks web server (http://molevol.cmima.csic.es/castresana/Gblocks_server.html). We confirmed the phylogenetic position of *Heteroplacidium* sp. represented by our three specimens by subjecting the alignment to a maximum likelihood (RAxML) analysis; nodal support was assessed using 1000 bootstrapping pseudoreplicates with RAxML-HPC v. 8.2.6 (Stamatakis 2014) and MrBayes v.3.2.6 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003) on the CIPRES Science Gateway (http://www.phylo.org). In the ML and Bayesian analyses, substitution models for ITS were estimated using jModelTest-2.1.9 (Guindon & Gascuel 2003, Darriba & al. 2012). Based

Table 1. Specimens and sequences of *Endocarpon*, *Heteroplacidium*, and *Placidium* used in the phylogenetic analysis

Species	Voucher	Origin	GenBank No.
E. adscendens	CG671	Switzerland	KF959777
E. pusillum	CG470	—	JQ927447
E. tenuissimum	Lendemer 29447	USA	KM371592
	Lendemer 27013	USA	KM371593
H. acervatum	LI 271015	Spain	GU228954
	M. Prieto 399	Spain	GU228955
H. compactum	M. Prieto 1701	Spain	GU228952
	M. Prieto 1607	Spain	GU228949
	XL2017117	China	MH930459*
	ALS2018002	China	MH930460*
	ALS2018036	China	MH930461*
H. congestum	LI 552268	USA	GU228950
	LI 297536	USA	GU228951
H. divisum	LI 218428	Italy	GU228953
P. pilosellum	M. Prieto 3	Spain	GU228993
	VEL_PLG	Slovakia	KY981585
P. podolepis	LI 297365	Argentina	GU228956
P. squamulosum	M. Prieto 336	Spain	GU228994

* = sequences newly generated for this study by the authors

on these results, we used the TrN+I+G model with 1000 pseudoreplicates in the ML analysis, and two parallel Markov chain Monte Carlo (MCMC) runs were performed in MrBayes, each using 8 million generations and sampling every 1000 steps. A 50% majority-rule consensus tree was generated from the combined sampled trees of both runs after discarding the first 25% as burn-in. Tree files were visualized with FigTree v.1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

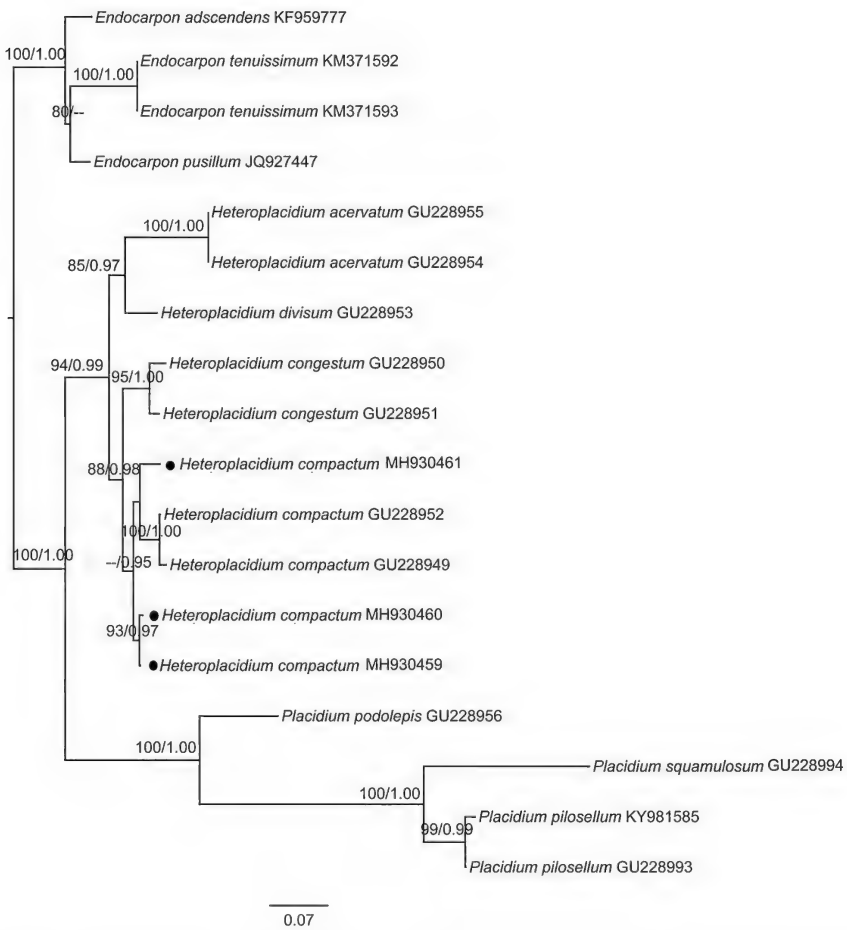

FIG. 2. The maximum likelihood tree of *Endocarpon*, *Heteroplacidium*, and *Placidium* species based on ITS sequences. The numbers in each node represent bootstrap support (BS) and posterior probability (PP) values. Bootstrap values ≥75 and posterior probability values ≥0.95 were plotted on the branches of the RAxML tree. Except for the three samples of *Heteroplacidium compactum* marked by the solid circle '●', all other sequences were downloaded from GenBank. Scale bar = 0.07 substitution per site.

Phylogenetic and species delimitation analyses

The aligned matrix contained 455 unambiguous ITS nucleotide positions. The phylogenetic tree (Fig. 2) included 10 taxa representing three genera within *Verrucariaceae*. *Heteroplacidium* formed a well-supported monophyletic clade (BS = 94, PP = 0.99) with the distinct species clearly separated. Our three Chinese specimens all grouped with Spanish sequences of *H. compactum*.

Taxonomy

Heteroplacidium compactum (A. Massal.) Gueidan & Cl. Roux,
Bull. Inf. Ass. Franç. Lichén. 33(1): 25, 2008. Fig. 3

Thallus areolate, areoles 0.6–1.8 mm diam., angular to rounded, flat to slightly convex; upper surface dark brown to black, dull to somewhat shiny.

Thallus layer ≤300 µm thick; upper cortex paraplectenchymous, 12.5–37.5 µm thick; algal layer 62.5–150 µm thick, cells 12.5–17.5 µm diam; medulla 112.5–187.5 µm thick; lower cortex paraplectenchymous, 10–12.5 µm thick or not clearly delimited. Rhizohyphae simple, intersecting,

Fig. 3. *Heteroplacidium compactum* (HMAS-L 140900): A. Habit of thallus; B. Anatomical structure of thallus; C. Perithecia in thallus; D. Ascospores; E. Separate areole with abundant rhizines tangling with soil particles. Scale bars: A = 1 mm; B = 20 µm; C = 100 µm; D = 50 µm; E = 0.5 mm.

4.5–7.5 µm diam. Perithecia nearly spherical, 300–500 µm diam, exciple, pale darkening with age. Asci clavate, 52.5–62.5 × 15–17.5 µm. Ascospores simple, ellipsoid, 12.5–17.5 × 10–12.5 µm. Pycnidia not seen.

Specimens examined. **China. Inner Mongolia**: 39°28′22″N 101°04′04″E, 1563 m alt., on sand, 5 June 2018, D.L. Liu & al. ALS2018002 (HMAS-L 0141672; GenBank MH930460); 39°32′31″N 101°06′34″E, 1478 m alt., on sand, 5 June 2018, D.L. Liu et al. ALS2018036 (HMAS-L 0141671; GenBank MH930461). **Ningxia**: **Zhongwei City**, Cui Liu Gou. 37°24′35″N 104°35′09″E, 1577 m alt., on sand, 15 July 2017, D.L. Liu & R.D. Liu XL2017117 (HMAS-L 140900; GenBank MH930459).

Distribution: *Heteroplacidium compactum* has previously been reported from Asia (Mongolian People's Republic; Pakistan), Europe, and northern Africa (Breuss 1994; Prieto & al. 2010a). In China, it is now known from arid desert areas in Inner Mongolia and Ningxia.

Discussion

The characters of Chinese *H. compactum* are essentially similar to the previous descriptions (Breuss 1994; Prieto & al. 2010a) in the areolate thallus, dark brown upper surface, paraplectenchymous upper and lower cortex, and the shape and size of the perithecia and asci. Among the differences noted are a thinner thallus (300 µm versus 600 µm) and broader ascospores (10–12.5 µm versus 8-10 µm). Furthermore, pycnidia were not seen in the Chinese materials.

In the arid desert area of China, *Endocarpon* is quite common and comprises a key component of biological soil crust communities (BSC). The biodiversity reported for these Chinese BSCs includes several newly described *Endocarpon* species (Yang & Wei 2008, Zhang & al. 2017a) with the predominant species, *E. pusillum*, studied physiologically (Zhang & Wei 2011), ecologically (Ding & al. 2013), and genomically (Wang & al. 2014, 2015, Li & Wei 2016, Zhang & al. 2017b). *Heteroplacidium* (with *Dermatocarpon*-type pycnidia) is clearly different from *Endocarpon* (with *Endocarpon*-type pycnidia).

The most similar genus to *Heteroplacidium* is *Placidium* A. Massal., which is also characterized by *Dermatocarpon*-type pycnidia, but the two genera have differently sized thallus squamules (*Heteroplacidium* 0.3–3 mm diam; *Placidium* 2–8(–15) mm diam). In the phylogenetic tree (Fig. 2), *Heteroplacidium* is most closely related to *Placidium*, clustering in a well-supported clade (BS = 100, PP = 1) and relatively distant from *Endocarpon*.

Acknowledgments

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Literature cited

Breuss O. 1994. Über einige wenig bekannte *Verrucaria*-Arten (Lichenes, *Verrucariaceae*). Österreichische Zeitschrift für Pilzkunde 3: 15–20.

Breuss O. 1996. Ein verfeinertes Gliederungskonzept für *Catapyrenium* (lichenisierte Ascomyceten, *Verrucariaceae*). Annalen Naturhistorischen Museums in Wien 98 B Suppl.: 35–50.

Castresana J. 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Molecular Biology and Evolution 17(4): 540–552. https://doi.org/10.1093/oxfordjournals.molbev.a026334

Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9(8): 772. https://doi.org/10.1038/nmeth.2109

Ding LP, Zhou QM, Wei JC. 2013. Estimation of *Endocarpon pusillum* carbon budget in the Tengger Desert based on its photosynthetic rate. Science China Life Sciences 56(9): 848–855. https://doi.org/10.1007/s11427-013-4526-9

Gueidan C, Roux C, Lutzoni F. 2007. Using a multigene phylogenetic analysis to assess generic delineation and character evolution in *Verrucariaceae* (*Verrucariales*, *Ascomycota*). Mycological Research 111(10): 1145–1168. https://doi.org/10.1016/j.mycres.2007.08.010

Gueidan C, Savić S, Thüs H, Roux C, Keller C, Tibell L, Prieto M, Heiðmarsson S, Breuss O, Orange A, Fröberg L, Wynns AA, Navarro-Rosinés P, Krzewicka B, Pykälä J, Grube M, Lutzoni F. 2009. Generic classification of the *Verrucariaceae* (*Ascomycota*) based on molecular and morphological evidence: recent progress and remaining challenges. Taxon 58: 184–208. https://doi.org/10.1002/tax.581019

Guindon S, Gascuel O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Systematic Biology 52(5): 696–704. https://doi.org/10.1080/10635150390235520

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Serie 41: 95–98.

Huelsenbeck JP, Ronquist F. 2001. MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17(8): 754–755. https://doi.org/10.1093/bioinformatics/17.8.754

Knudsen K, Breuss O, Kocourková J. 2014. A new lichenicolous *Heteroplacidium* (*Verrucariaceae*) from the deserts of southern California. Opuscula Philolichenum 13: 26–33.

Li H, Wei JC. 2016. Functional analysis of thioredoxin from the desert lichen-forming fungus, *Endocarpon pusillum* Hedwig, reveals its role in stress tolerance. Scientific Reports 6: 27184. https://doi.org/10.1038/srep27184

Prieto M, Aragón G, Martínez I. 2010a. The genus *Catapyrenium* s. lat. (*Verrucariaceae*) in the Iberian Peninsula and the Balearic Islands. Lichenologist 42(6): 637–684. https://doi.org/10.1017/S0024282910000319

Prieto M, Martínez I, Aragón G, Otálora MAG, Lutzoni F. 2010b. Phylogenetic study of *Catapyrenium* s. str. (*Verrucariaceae,* lichen-forming *Ascomycota*) and related genus *Placidiopsis*. Mycologia 102(2): 291–304. https://doi.org/10.3852/09-168

Rogers SO, Bendich AJ. 1988. Extraction of DNA from plant tissues. A6: 1–10, in: Gelvin SB, Schilperoort RA (eds). Plant Molecular Biology Manual. Kluwer Academic Publishers, Boston. https://doi.org/10.1007/978-94-017-5294-7_6

Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA & al. 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. Proceedings of the National Academy of Sciences of the United States of America 109(16): 6241–6246. https://doi.org/10.1073/pnas.1117018109

Stamatakis A. 2014. RAxML Version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30: 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Talavera G, Castresana J. 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Systematic Biology 56(4): 564–577. https://doi.org/10.1080/10635150701472164

Thompson J, Higgins D, Gibson T. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research 22(22): 4673–4680. https://doi.org/10.1093/nar/22.22.4673

Vilgalys R, Hester M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology 172: 4238–4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990

Wang YY, Liu B, Zhang XY, Zhou QM, Zhang T, Li H, Yu YF, Zhang XL, Hao XY, Wang M, Wang L, Wei JC. 2014. Genome characteristics reveal the impact of lichenization on lichen-forming fungus *Endocarpon pusillum* Hedwig (*Verrucariales, Ascomycota*). BMC Genomics 15:34 [18 p.]. https://doi.org/10.1186/1471-2164-15-34

Wang YY, Zhang XY, Zhou QM, Zhang XL, Wei JC. 2015. Comparative transcriptome analysis of the lichen-forming fungus *Endocarpon pusillum* elucidates its drought adaptation mechanisms. Science China Life Sciences 58: 89–100. https://doi.org/10.1007/s11427-014-4760-9

White T, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. 315–322, in: MA Innis & al. (eds). PCR protocols: a guide to methods and applications. San Diego, CA. Academic Press. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Yang J, Wei JC. 2008. The new lichen species *Endocarpon crystallinum* from semiarid deserts in China. Mycotaxon 106: 445–448.

Zhang T, Wei JC. 2011. Survival analyses of symbionts isolated from *Endocarpon pusillum* Hedwig to desiccation and starvation stress. Science China Life Sciences 54(5): 480–489. https://doi.org/10.1007/s11427-011-4164-z

Zhang T, Liu M, Wang YY, Wang ZJ, Wei XL, Wei JC. 2017a. Two new species of *Endocarpon* (*Verrucariaceae, Ascomycota*) from China. Scientific Reports 7:7193 [11 p.]. https://doi.org/10.1038/s41598-017-07778-5

Zhang YL, Li H, Wang YY, Wei JC. 2017b. The calcium-binding protein EpANN from the lichenized fungus *Endocarpon pusillum* enhances stress tolerance in yeast and plants, Fungal Genetics and Biology 108: 36–43. https://doi.org/10.1016/j.fgb.2017.09.003

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Geopora ahmadii sp. nov. from Pakistan

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Abstract—A new species, *Geopora ahmadii,* is described and illustrated based on material from Punjab, Pakistan. This species is characterized by sessile, cup- to saucer-shaped, partly immersed apothecia with whitish to grayish hymenial surfaces; broad ellipsoid, mostly uniguttulate ascospores; and brown excipular hairs. Phylogenetic analyses of the nrDNA ITS region with maximum parsimony, maximum likelihood, and Bayesian inference methods reveal that *G. ahmadii* is distinct from other described *Geopora* species. A collection previously identified as *Geopora arenosa* from Rawalakot, Pakistan, likely represents a second locality of *G. ahmadii*.

Key words—*Ascomycota,* hypogeous fungi, *Pezizales, Pyronemataceae,* taxonomy

Introduction

Geopora Harkn. (*Pyronemataceae*, *Pezizales*) is characterized by (1) ascomata that occur entirely or partially below ground and are covered with brown, septate excipular hairs; (2) a whitish, grayish, or yellowish-gray hymenium; and (3) smooth, mostly uniguttulate ascospores. Because ascomata appear infrequently and are hypogeous at some developmental

stage, *Geopora* specimens are infrequently collected. Species delimitation is additionally challenging due to the scarcity of distinctive morphological characters, with measurement ranges overlapping among species (Tamm & al. 2010, Guevara-Guerrero & al. 2012, Flores-Rentería & al. 2014).

Identification of *Geopora* species has relied primarily on ascospore shape and size, position of apothecia in the ground, and the length of excipular hairs (Burdsall 1965, 1968; Tamm & al. 2010; Flores-Rentería & al. 2014). Molecular analyses by Tamm & al. (2010) showed that well supported clades are not congruent with morphological species concepts. The combination of molecular and morphological data is considered the most reliable approach to define species in this genus (Southworth & Frank 2011, Guevara-Guerrero & al. 2012, Flores-Rentería & al. 2014).

Perry & al. (2007) studied the phylogenetic relationships of *Pyronemataceae*. Using LSU ribosomal DNA (rDNA) sequence data, they suggested that *Geopora* is monophyletic. Hansen & al. (2013) determined that *Geopora* was sister to *Tricharina* in the larger *Scutellinia–Trichophaea* lineage. Neither study included species representing *Phaeangium* Pat. or *Picoa* Vittad. Stielow & al. (2013) suggested that *Geopora* was paraphyletic including species of both *Phaeangium* and *Picoa*. They also found that *Geopora pellita* (Sacc.) T. Schumach. was phylogenetically isolated from other *Geopora* species and created the new genus *Hoffmannoscypha* Stielow & al. to accommodate this taxon.

During our studies of ectomycorrhizal fungi, we found *Geopora* specimens growing in groups on damp soil in Punjab, Pakistan. Molecular phylogenetic analysis of the ITS rDNA region combined with morphological evaluation support the recognition of our collections as a new species. This species is described, illustrated, and compared with other *Geopora* species.

Materials & methods

Morphological studies

Ascomata were collected and dried in a food dehydrator at 39 °C for 7–9 hours. Shape, texture, and dimensions of important characters were recorded from fresh ascomata. Colors were compared to the Munsell Soil Color Charts (1975). Dried voucher specimens are deposited at the University of the Punjab Herbarium, Lahore, Pakistan (LAH) and the Farlow Herbarium, Harvard University, Cambridge, MA, USA (FH).

Sections of specimens were mounted in water and Congo red in ammonia (0.3% in commercial ammonia cleaner) to increase contrast for microscopic observations.

Micromorphological analysis, photographs, and measurements were made

using an Olympus Bx40 light microscope with Olympus XC50 digital camera and Microsuite Special Edition software 3.1. Sections were made using a freezing microtome. Measurements include the typical range with extremes given in parentheses. Q values (length/width ratios) are given for ascospores.

DNA extraction, PCR amplification, DNA sequencing

Genomic DNA was extracted from a small piece of an ascoma by a modified CTAB method (Gardes & Bruns 1993). The internal transcribed spacer region (ITS1+5.8S+ITS2) of the nuclear ribosomal RNA gene was amplified using the primer pair ITS1F and ITS4 (White & al. 1990, Gardes & Bruns 1993) and the RED Extract-N-Amp PCR ReadyMix. PCR cycling parameters comprised initial denaturation (94° C for 1 min), 35 cycles (94 °C for 1 min, 53 °C for 1 min, and 72 °C for 1 min), and final extension 72 °C (8 min). Amplified PCR products were outsourced to Macrogen, (Seoul, Republic of Korea) for purification and bidirectional sequencing.

Sequence alignment & phylogenetic analyses

Geopora sequences downloaded from GenBank included those studied by Tamm & al. (2010) and sequences representing recently described species—*G. cercocarpi* D. Southw. & J.L. Frank, *G. gilkeyae* (Burds.) G. Guevara & al., *G. pinyonis* Flores-Rent. & Gehring, and *G. tolucana* G. Guevara & al. (Flores-Rentería & al. 2014, Guevara-Guerrero & al. 2012, Southworth & Frank 2011). *Tarzetta catinus* (Holmsk.) Korf & J.K. Rogers and *Trichophaea hybrida* (Sowerby) T. Schumach. (*Pyronemataceae*, *Pezizales*) were selected as the outgroup because they are closely related to *Geopora* (Perry & al. 2007).

Manually edited sequences were assembled in BioEdit v7.2.6 (www.mbio.ncsu.edu/bioedit/bioedit.html). All sequences were trimmed with the conserved motifs 5′-(...GAT)CATTA– and –GACCT(CAAA...)-3′ (Dentinger & al. 2011), and the alignment portions between them were included in the analysis. Sequences retrieved from NCBI GenBank were aligned by Muscle v3.7 (Edgar 2004) with default parameters using Molecular Evolutionary Genetics Analysis (MEGA) software (Tamura & al. 2011).

Maximum parsimony (MP) analysis was performed with PAUP 4.0b on XSEDE (Swofford 1991), available on the Cipres Gateway v3.3 (Miller & al. 2010). All characters were equally weighted and gaps were treated as missing data. The heuristic search option with tree-bisection-reconnection (TBR) branch swapping and 1000 random sequence additions were used to infer trees. Clade robustness was assessed using a bootstrap analysis with 500 replicates (Felsenstein 1985). A maximum likelihood (ML) analysis was carried out with RAxML XSEDE on the Cipres Gateway, using the general time-reversible (GTR) model of nucleotide substitution (Stamatakis & al. 2008). Nodal support was determined from 1000 bootstrap replicates.

Bayesian analysis was done with a Markov chain Monte Carlo (MCMC) coalescent approach implemented in Beast v1.8.2 (Drummond & Rambaut 2007),

TABLE 1. *Geopora* isolates and outgroup included in phylogenetic analyses
[Clade designations sensu Tamm & al. 2010]

ORIGINAL ID	CLADE	COUNTRY	VOUCHER	GENBANK	REFERENCE
Tarzetta catinus		Estonia	TAAM 192291	FM206478	Tamm & al. 2010
Trichophaea hybrida		Estonia	TAAM 192334	FM206477	Tamm & al. 2010
G. cercocarpi		USA, OR	SOC 1590	HQ283090	Southworth & Frank 2011
G. cercocarpi		USA, OR	SOC 1590	NR121491	Southworth & Frank 2011
G. pinyonis		USA, AR	DGB 27586	KF768653	Flores-Rentería & al. 2014
G. pinyonis		USA, AR	DGB 27586	KF768652	Flores-Rentería & al. 2014
G. tolucana		Mexico	ITCV 1081	HQ184961	Guevara-Guerrero & al. 2012
G. tolucana		Mexico	ITCV 1081	HQ184960	Guevara-Guerrero & al. 2012
G. ahmadii	**IX**	**Pakistan**	**MSM#0091 [T]**	**KY805995**	**This paper**
G. ahmadii	**IX**	**Pakistan**	**MSM#00163**	**KY805996**	**This paper**
G. arenicola	IX	Estonia	TAAM 192329	FM206473	Tamm & al. 2010
G. sp.	IX	Estonia	TAAM 192324	FM206471	Tamm & al. 2010
G. arenicola	IX	Estonia	TAAM 192330	FM206472	Tamm & al. 2010
G. foliacea	IX	Estonia	TAAM 192323	FM206470	Tamm & al. 2010
Ectomycorrhizal	X	France	ECM 2	AJ410862	El Karkouri & al. 2004
Ectomycorrhizal	X	France	ECM 95	AJ410865	El Karkouri & al. 2004
Ectomycorrhizal uncultured	X	Spain	Riv-4	EF484934	Rincón & al. 2007
G. cf. *sepulta*	X	Estonia	TAAM 113526	FM206476	Tamm & al. 2010
G. cooperi	X		101GA	AF387651	Gutierrez & al. (unpubl.)
G. cooperi	X		108GC	AF387649	Gutierrez & al. (unpubl.)
G. cooperi	X		109GC	AF387650	Gutierrez & al. (unpubl.)
G. gilkeyae		USA, CA	src515	DQ974731	Smith & al. 2007
G. arenicola	VIII	Estonia	TAAM 188666	FM206449	Tamm & al. 2010
G. arenicola	VIII	Estonia	TAAM 188339	FM206446	Tamm & al. 2010
G. arenicola	VIII	Estonia	TAAM 117708	FM206460	Tamm & al. 2010
G. arenicola	VIII	Estonia	TAAM 188293	FM206440	Tamm & al. 2010
G. arenicola	VIII	Estonia	TAAM 188292	FM206439	Tamm & al. 2010
G. arenicola	VIII	Estonia	TAAM 135060	FM206433	Tamm & al. 2010
G. arenicola	VIII	Estonia	TAAM 116784	FM206462	Tamm & al. 2010
G. sepulta	VII	Estonia	TAAM 192311	FM206432	Tamm & al. 2010

Original ID	Clade	Country	Voucher	GenBank	Reference
G. sepulta	VII	Estonia	TAAM 192333	FM206431	Tamm & al. 2010
G. foliacea	V	Finland	H RS-34685	FM206428	Tamm & al. 2010
G. foliacea	V	Finland	H RS-29584	FM206424	Tamm & al. 2010
G. cervina	V	Finland	H RS-17984	FM206426	Tamm & al. 2010
G. cervina	VI	Estonia	TAAM 192232	FM206420	Tamm & al. 2010
G. tenuis	VI	Estonia	TAAM 192302	FM206429	Tamm & al. 2010
G. sp.	VI	Tajikistan	TAAM 116668	FM206475	Tamm & al. 2010
G. tenuis	IV	Estonia	TAAM 188326	FM206397	Tamm & al. 2010
G. tenuis	IV	Finland	H RS-09584	FM206402	Tamm & al. 2010
G. tenuis	IV	Estonia	TAAM 188331	FM206396	Tamm & al. 2010
G. cervina	IV	Estonia	TAAM 192293	FM206401	Tamm & al. 2010
G. cervina	III	Estonia	TAAM 117479	FM206413	Tamm & al. 2010
G. cervina	III	Finland	H RS-07186	FM206406	Tamm & al. 2010
G. cervina	III	Estonia	TAAM 117884	FM206409	Tamm & al. 2010
G. cervina	III	Estonia	TAAM 192321	FM206410	Tamm & al. 2010
G. arenicola	III	Estonia	TAAM 117952	FM206412	Tamm & al. 2010
G. cervina	II	Estonia	TAAM 188304	FM206417	Tamm & al. 2010
G. cervina	II	Estonia	TAAM 117898	FM206419	Tamm & al. 2010
G. arenicola	II	Estonia	TAAM 188517	FM206418	Tamm & al. 2010
G. cervina	I	Finland	H RS-06986	FM206387	Tamm & al. 2010
G. cervina	I	Estonia	TAAM 188655	FM206390	Tamm & al. 2010
G. cervina	I	Estonia	TAAM 117854	FM206391	Tamm & al. 2010
G. cervina	I	Estonia	TAAM 117659	FM206389	Tamm & al. 2010

with an uncorrelated lognormal relaxed clock for rate variation across the tree. A Bayesian skyride coalescent tree GMRF prior with the GTR+I+G model of nucleotide substitution was used in all simulations, with a randomly generated starting tree. Four independent runs of 10 million generations were undertaken. Tracer v1.6.0 (Drummond & Rambaut 2007) was used to check the effective sample size (ESS), and burn-in values were adjusted to achieve a net ESS of at least 200. Upon removal of a portion of each run as burn-in, log files and trees files were combined in LogCombiner v.1.8.2. Finally, a consensus tree (0% burn-in) was generated using TreeAnnotator v1.8.2 and visualized in FigTree v1.4.2.

Sequences of *Geopora ahmadii* generated during this study were submitted to GenBank. Accession numbers for the sequences downloaded from GenBank and those sequenced during this study are given in Table 1.

Taxonomy

Geopora ahmadii Saba, T. Ashraf, Khalid & Pfister, **sp. nov.** FIGS 1, 2

MB 822666

Differs from *Geopora arenicola* by its partly immersed apothecia that are larger in diameter, cupulate when young but saucer-shaped when older, by its broadly ellipsoid ascospores, and by its ITS sequence with 49–52 autapomorphies.

TYPE: Pakistan, Punjab, Lahore, University of the Punjab, Department of Botany, Botanical Garden, 31°29′56″N 74°17′57″E, 6 March 2009, leg. M. Saba, T. Ashraf & A.N. Khalid, MSM#0091 (**Holotype**, LAH 310019; GenBank KY805995).

ETYMOLOGY: Named in honor of Dr. Sultan Ahmad (1910–1983), eminent pioneering mycologist in Pakistan.

APOTHECIA partly immersed in soil, sessile, fleshy, cup-shaped at early stages and saucer-shaped at older stages when becoming thin, flat-discoid, ≤15–25 mm in diameter and 4 mm deep when fresh; when dry shrinking to 6–10 mm diam; disc grey and smooth when fresh, whitish beige to whitish grey when

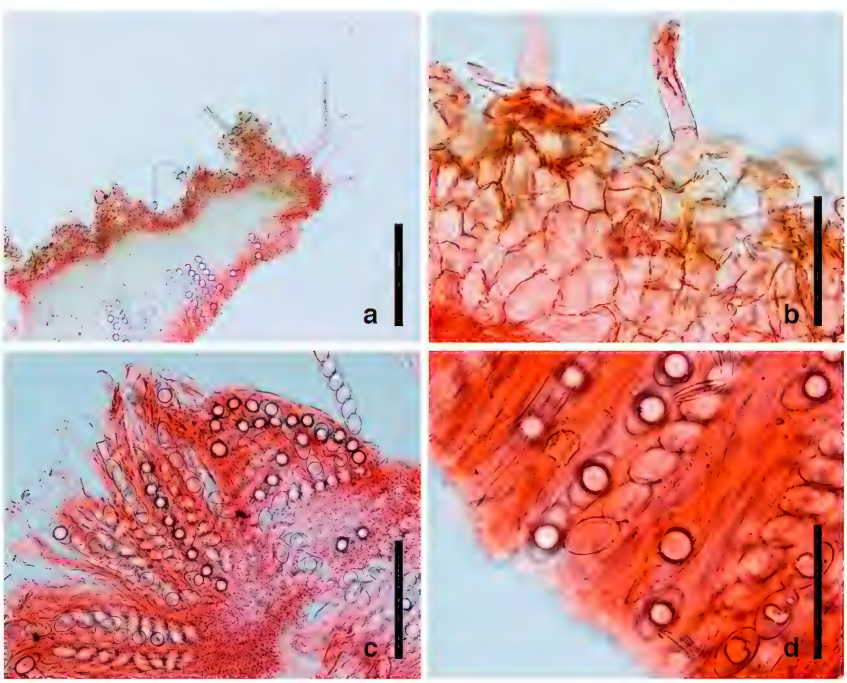

FIG. 1. *Geopora ahmadii* (holotype, LAH310019). a. Section of ascoma showing hymenium, subhymenium, excipulum, and excipular hairs; b. Excipular cells with single excipular hair; c. Asci and paraphyses; d. Asci and paraphyses, with detail of an ascospore with a single guttule (insert). Scale bars: a = 200 µm; b, d = 50 µm; c = 100 µm.

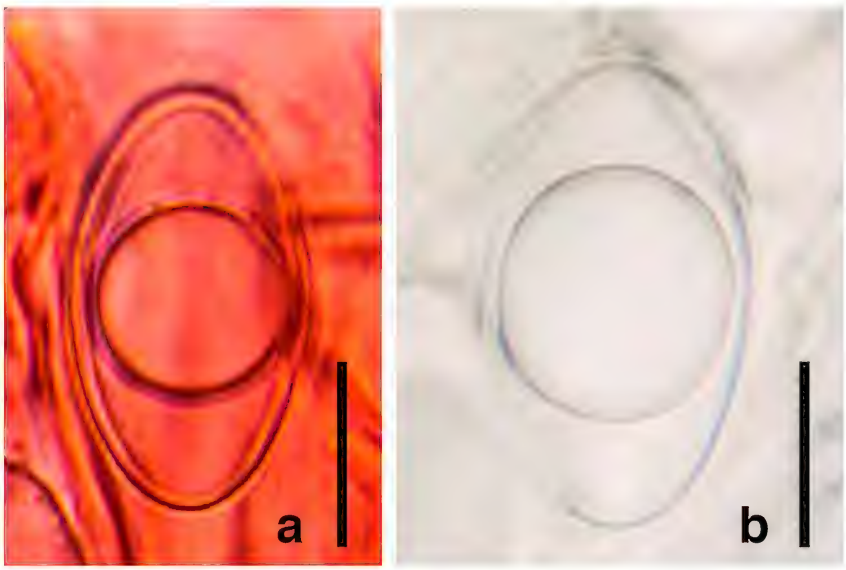

Fig. 2. *Geopora ahmadii* (holotype, LAH310019). Ascospores, each with a single guttule: a. Mounted in Congo red in ammonia; b. Mounted in water. Scale bars = 10 µm.

rehydrated; receptacle dark brown, warted and hairy. Margin wavy at young stages, splitting into 5–6 lobes at older stages.

Hymenium 180–220 µm thick. Asci cylindrical, J–, (175–)210–250(–340) × 15–21 µm, with 8 ascospores. Subhymenium dense textura intricata, dark brown, compact. Ascospores uniseriate, broadly ellipsoid, with a single guttule, and smaller guttules at the poles, 19–26.0 × (11–)12–15 µm, Q = 1.68–1.94; wall 1.0–1.5 µm thick, hyaline. Paraphyses slender, hyaline, broadly clavate, 7–10 µm diam. at the tip, 5–6 µm diam. in the middle, septate. Ectal excipulum 75–87(–115) µm thick, dark brown of textura globulosa to textura angularis, cells round to irregular to polygonal, 16–30 × 13–16 µm, walls brown, 1–2 µm thick (especially thicker in the outermost cells), outer cells aggregated to form warts. Medullary excipulum (40–)50–70(–80) µm thick; dense textura intricata, cells appearing angular to irregular to roundish, becoming smaller towards the subhymenium, cells 8.5–20.5 × 5–12 µm. Hairs arising from globose ectal excipular cells, septate and forming a mat of brown hyphae holding soil particles. When young thin, straight, light brown, smooth, (6–)7.5–10.0(–16) µm diam.; with age branched, twisted and curved, dark brown, ≤(40–)50–70(–80) µm diam., with dark granular walls and granular content; hair walls 0.8–1.5(–2) µm thick.

Additional material studied: **PAKISTAN**, **Punjab**, **Lahore**, University of the Punjab, Botanical Garden, 31°29′56″N 74°17′57″E, 16 June 2011, MSM#00160 (LAH 310099!); 5 May 2013, leg. M. Saba, T. Ashraf & A.N. Khalid, MSM#00163 (FH 01142414; GenBank KY805996).

Molecular analyses of the genus *Geopora*

Initial BLAST analysis of the *G. ahmadii* ITS sequence showed a maximum identity of 84% with collection TAAM 192330 (GenBank accession number FM206472). This collection belongs to clade IX (sensu Tamm & al. 2010) but was identified based on morphology as *G. arenicola* (Lév.) Kers. Our data matrix included 53 isolates, all representing identified *Geopora* species except for three unidentified ectomycorrhizal isolates (El Karkouri & al. 2004, Rincón & al. 2007) and the two outgroup sequences (Table 1). The final aligned data matrix included 722 characters, of which 272 were constant and 351 were parsimony-informative.

Our MP and ML analyses (Fig. 3) largely agree. One exception is the placement of *G. gilkeyae* (Burds.) Guevara & al. (as *"Geopora cooperii* var. *gilkeyi"* in Smith & al. 2007) and *G. tolucana* Guevara & al. In the MP topology (not shown), these species are sister taxa and form a branch basal to all other *Geopora* species (sensu Tamm & al. 2010). However, there is no bootstrap support for this placement. Also, the placement of *G. gilkeyae* in the ML analysis is unresolved (no bootstrap support). In the three analyses (MP, ML, Bayesian), a number of the basal branches lack support, and phylogenetic reconstructions based on ITS alone cannot resolve relationships of *Geopora* at deeper nodes.

Clades I through X (sensu Tamm & al. 2010) are recognized here with high support from MP, ML, and Bayesian analyses (Figs 3, 4). *Geopora ahmadii* is inferred in *Geopora* clade IX from Tamm & al. (2010) with maximum support. The morphological characters of *G. ahmadii* (fruit-body features, ascospore dimensions, Q ascospore values) are consistent with those of clade IX as given by Tamm & al. (2010). The species is recovered as sister to "*Geopora* sp. b" (sensu Schumacher 1979; defined in the study of Tamm & al. 2010) in clade IX. *Geopora* sp. b is known only from northern Europe, with records from Estonia (Tamm & al. 2010) and Norway (Schumacher 1979).

Discussion

Tamm & al. (2010) reviewed the history of the genus *Geopora* and confirmed that delimitations of species within *Geopora* are difficult. All

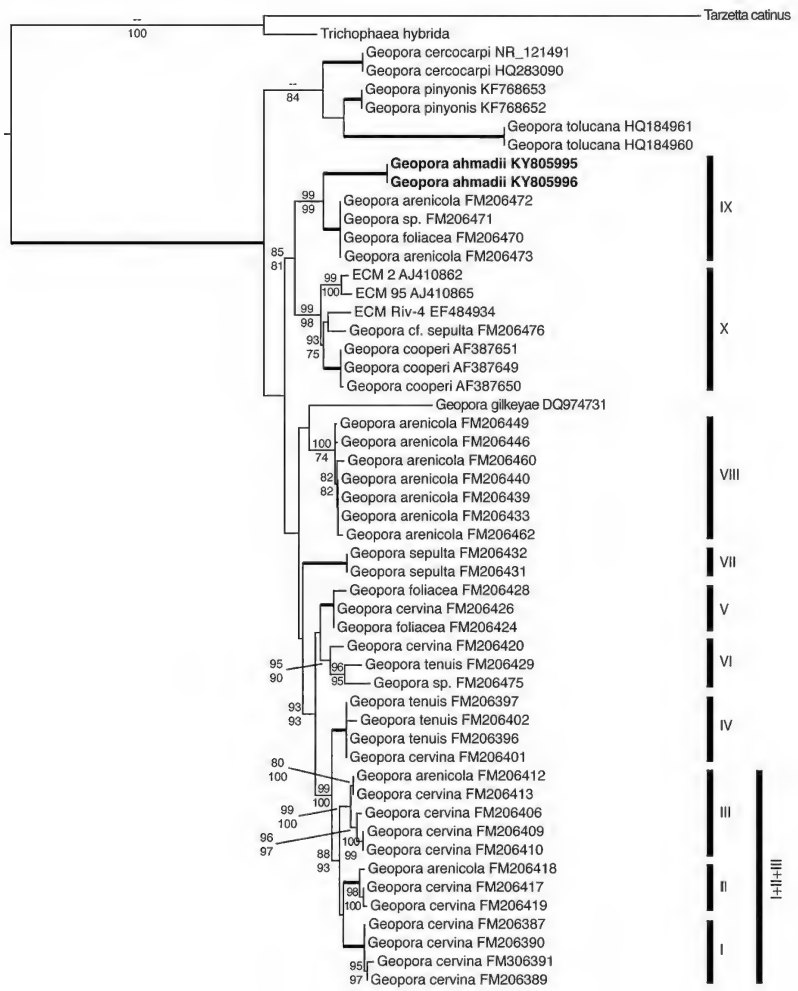

FIG. 3. Phylogeny of *Geopora* species produced from maximum likelihood (ML) analysis of the ITS rDNA dataset. The best-scoring ML tree (log likelihood of –6541.843463) is shown. Only bootstrap values >70% are given. Maximum parsimony (MP) bootstrap values are given above the branches; ML bootstrap values are below the branches. Branches in bold have maximum support.

known taxa are ectomycorrhizal (Tedersoo & al. 2006) but we have not been able to determine the mycorrhizal associate(s) for our new species. The ectomycorrhizal host could be *Pinus roxburghii,* which grows near the site where this species has been repeatedly collected in the Botanical Garden

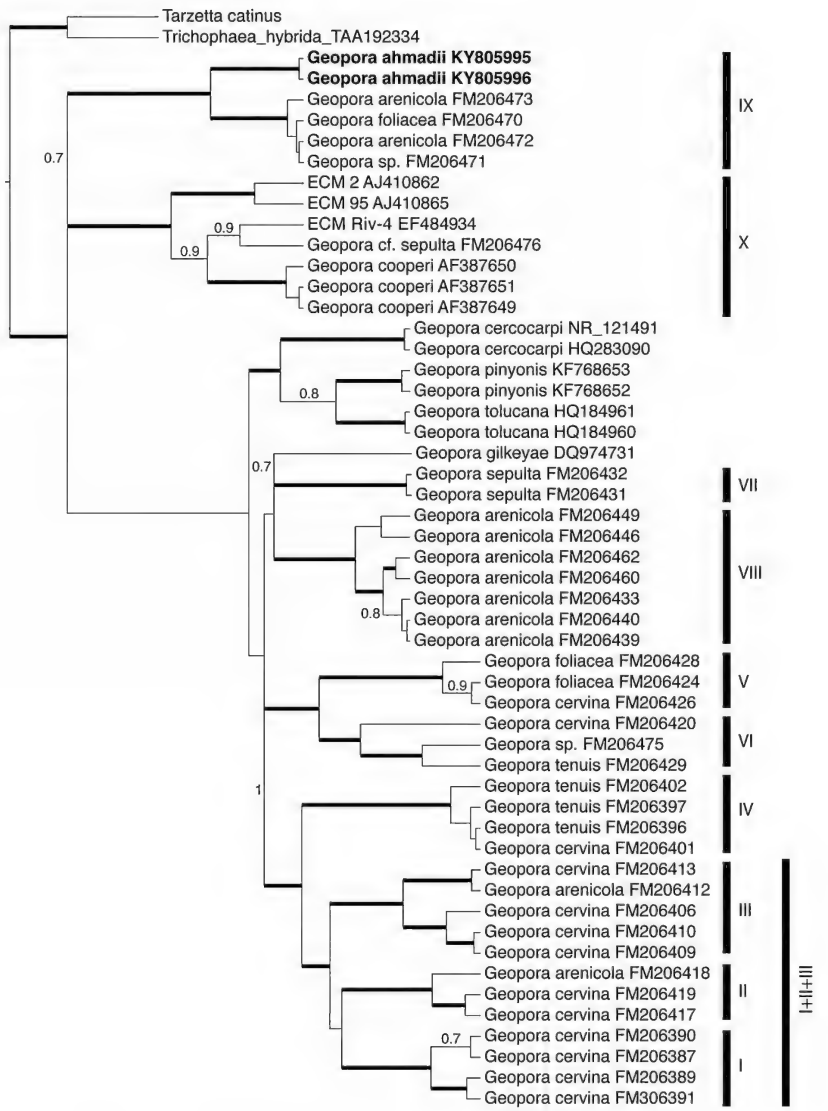

Fig. 4. Phylogeny of *Geopora* species produced from Bayesian inference of the ITS rDNA dataset. Only posterior probabilities ≥0.9 are shown. Branches in bold have maximum support.

of the Department of Botany, University of the Punjab, Lahore, Pakistan. Associations of the new species should be evaluated with sequences from root tip samples.

Geopora ahmadii is distinct from other *Geopora* species based on apothecial shape, partial immersion of the apothecium in the soil, hymenial color, and ascospore size. Other partly immersed species are *G. tenuis* (Fuckel) T. Schumach. and *G. cervina* (Velen.) T. Schumach. (Yao & Spooner 1996), which are distinguished by different spore sizes ((20.3–)21.4(–23.0) × (10.8–)11.6(–12.1) for *G. tenuis;* (20.8–)23.8(–26.2) × (10.8–)12.1(–14.2) µm for *G. cervina;* Tamm & al. 2010) and placement in different clades in our analyses. The hymenial color in *G. ahmadii* is similar to *G. sepulta* (Fr.) Korf & Burds., which differs by complete immersion in the soil and placement in another clade.

Our molecular analyses place *G. ahmadii* in clade IX sensu Tamm & al. (2010). Its morphological characters—including the position and shape of the ascomata and ascospore dimensions—are consistent with placement among the other clade IX species. This clade includes specimens initially identified as *G. arenicola* and *G. foliacea* (Schaeff.) S. Ahmad. *Geopora arenicola*, as defined by Tamm & al. (2010), has a completely immersed ascoma. The name *G. foliacea* has been variously applied.

Once again, this study underscores the difficulties of using morphology for species delimitation in this group. Southworth & al. (2011) also noted the difficulty in using morphology to describe their new species, *G. cercocarpi,* citing its exclusive association with *Cercocarpus ledifolius* and that other *Geopora* specimens showed similar host fidelity. Unfortunately we do not know the associate of *G. ahmadii* but we plan to collect root tip samples under trees of its most likely candidate ectomycorrhizal associate, *Pinus roxburghii*.

One comment regarding *G. arenosa* (Fuckel) S. Ahmad: Ahmad (1978) transferred *Peziza arenosa* Fuckel [≡ *Humaria arenosa* (Fuckel) Fuckel] (Fuckel 1864, 1866, 1870) to *Geopora* based on a collection he studied from Rawalakot, Pakistan. According to his description, *G. arenosa* has globose to subglobose apothecia, a whitish gray hymenial surface, and ellipsoid ascospores measuring 20–24 × 13–14.5 µm. Yao & Spooner (1996) studied the type material of *G. arenosa* (Fuckel's Fungi Rhenani exsiccati, No. 1212, K—K(M) 69362, designated as lectotype by Yao & Spooner 2003), and their description contrasts with the material studied by Ahmad (1978) in ascospore shape and size (ellipsoid to fusoid, 27–30 × 13.5–15 µm in the *G. arenosa* type vs. ellipsoid, 20–24 × 13–14.5 µm in Ahmad's material). Because of these discrepancies, we believe that Ahmad's collection from Rawalakot represents a previous collection of our new species. We have not

been able to locate Ahmad's specimen and it is likely lost. It is not present in LAH and no sequence data are available for the Rawalakot material. Further collecting in Rawalakot may help to resolve the identity of Ahmad's species and to determine if it is indeed conspecific with *G. ahmadii*.

Acknowledgments

We are highly indebted to Higher Education Commission (HEC), Islamabad, Pakistan, for funding this project under Phase II, Batch I, Indigenous PhD fellowships program for 5000 scholars and through International Research Support Initiative Program (IRSIP). Finally, we thank Dr. Rosanne Healy and Dr. Matthew E. Smith (both of Dept. Plant Pathology, University of Florida, Gainesville, USA), and Nomenclature Editor Dr. Shaun B. Pennycook for critically reviewing the manuscript, which would have been a lot meagerer if it weren't for their valuable comments for improvement.

References

Ahmad S. 1978. Ascomycetes of Pakistan. Part 1. Biological Society of Pakistan, Lahore. Monograph No. 7.

Burdsall HH Jr. 1965. Operculate asci and puffing of ascospores in *Geopora* (*Tuberales*). Mycologia 57(3): 485–488. https://doi.org/10.2307/3756880

Burdsall HH Jr. 1968. A revision of the genus *Hydnocystis* (*Tuberales*) and of the hypogeous species of *Geopora* (*Pezizales*). Mycologia 60(3): 496–525. https://doi.org/10.2307/3757418

Dentinger BT, Didukh MY, Moncalvo JM. 2011. Comparing COI and ITS as DNA barcode markers for mushrooms and allies (*Agaricomycotina*). Plos One 6(9): e25081 [8 p.]. https://doi.org/10.1371/journal.pone.0025081

Drummond AJ, Rambaut A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evolutionary Biology 7(1): 214. https://doi.org/10.1186/1471-2148-7-214

Edgar, RC. 2004. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32: 1792–1797. https://doi.org/10.1093/nar/gkh340

El Karkouri K, Martin F, Mousain D. 2004. Diversity of ectomycorrhizal symbionts in a disturbed *Pinus halepensis* plantation in the Mediterranean region. Annals of Forest Science 61(7): 705–710. https://doi.org/10.1051/forest:2004069

Flores-Rentería L, Lau MK, Lamit LJ, Gehring CA. 2014. An elusive ectomycorrhizal fungus reveals itself: a new species of *Geopora* (*Pyronemataceae*) associated with *Pinus edulis*. Mycologia 106(3): 553–563. https://doi.org/10.3852/13-263

Fuckel L. 1864. Fungi Rhenani exsiccati, fasc. 13. No. 1212.

Fuckel L. 1866. Fungi Rhenani exsiccati. Cent. 12–17. N. 1101–1700. Hedwigia 5: 14–16.

Fuckel L. 1870. Symbolae mycologicae. Beiträge zur Kenntniss der Rheinischen Pilze. Jahrbücher des Nassauischen Vereins für Naturkunde 23–24. 459 p.

Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for *Basidiomycetes*: application to the identification of mycorrhizae and rusts. Molecular Ecology 2: 113–118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x

Guevara-Guerrero G, Stielow B, Tamm H, Cázares-Gonzalez E, Göker M. 2012. *Genea mexicana*, sp. nov., and *Geopora tolucana*, sp. nov., new hypogeous *Pyronemataceae* from Mexico, and the taxonomy of *Geopora* reevaluated. Mycological Progress 11(3): 711–724. https://doi.org/10.1007/s11557-011-0781-y

Hansen K, Perry BA, Dranginis AW, Pfister DH. 2013. A phylogeny of the highly diverse cup-fungus family *Pyronemataceae* (*Pezizomycetes*, *Ascomycota*) clarifies relationships and evolution of selected life history traits. Molecular Phylogenetics and Evolution 67: 311–335. https://doi.org/10.1016/j.ympev.2013.01.014

Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. 45–52, in: Proceedings of the Gateway Computing Environments Workshop (GCE) 14 Nov. 2010. New Orleans, Louisiana. https://doi.org/10.1109/GCE.2010.5676129

Munsell Soil Color Charts. 1975. Munsell Color Company. Baltimore, Maryland, USA.

Perry BA, Hansen K, Pfister DH. 2007. A phylogenetic overview of the family *Pyronemataceae* (*Ascomycota*, *Pezizales*). Mycological Research 111(5): 549–571. https://doi.org/10.1016/j.mycres.2007.03.014

Rincón A, de Felipe MR, Fernández-Pascual M. 2007. Inoculation of *Pinus halepensis* Mill. with selected ectomycorrhizal fungi improves seedling establishment 2 years after planting in a degraded gypsum soil. Mycorrhiza 18(1): 23–32. https://doi.org/10.1007/s00572-007-0149-y

Schumacher T. 1979. Notes on taxonomy, ecology, and distribution of operculate discomycetes (*Pezizales*) from river banks in Norway. Norwegian Journal of Botany 26: 53–83.

Smith ME, Douhan GW, Rizzo DM. 2007. Ectomycorrhizal community structure in a xeric *Quercus* woodland based on rDNA sequence analysis of sporocarps and pooled roots. New Phytologist 174(4): 847-863. https://doi.org/10.1111/j.1469-8137.2007.02040.x

Southworth D, Frank JL. 2011. Linking mycorrhizas to sporocarps: a new species, *Geopora cercocarpi*, on *Cercocarpus ledifolius* (*Rosaceae*). Mycologia 103(6): 1194–1200. https://doi.org/10.3852/11-053

Stamatakis A, Hoover P, Rougemont J. 2008. A rapid bootstrap algorithm for the RAxML web servers. Systematic Biology 57(5): 758–771. https://doi.org/10.1080/10635150802429642

Stielow B, Hensel G, Strobelt D, Makonde HM, Rohde M, Dijksterhuis J, Klenk HP, Göker M. 2013. *Hoffmannoscypha*, a novel genus of brightly coloured, cupulate *Pyronemataceae* closely related to *Tricharina* and *Geopora*. Mycological Progress 12(4): 675–686. https://doi.org/10.1007/s11557-012-0875-1

Swofford DL. 1991. PAUP: Phylogenetic Analysis Using Parsimony, version 3.1. Computer program distributed by the Illinois Natural History Survey, Champaign, Illinois, USA.

Tamm H, Põldmaa K, Kullman B. 2010. Phylogenetic relationships in genus *Geopora* (*Pyronemataceae*, *Pezizales*). Mycological Progress 9(4): 509–522. https://doi.org/10.1007/s11557-010-0659-4

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution 28(10): 2731–2739. https://doi.org/10.1093/molbev/msr121

Tedersoo L, Hansen K, Perry BA, Kjøller R. 2006. Molecular and morphological diversity of pezizalean ectomycorrhiza. New Phytologist 170: 581–596. https://doi.org/10.1111/j.1469-8137.2006.01678.x

White TJ, Bruns T, Lee SJWT, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. 315–322, in: MA Innis & al. (eds). PCR protocols: a guide to methods and applications. San Diego, CA. Academic Press. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Yao YJ, Spooner BM. 1996. Notes on British species of *Geopora*. Mycological Research 100(1): 72–74. https://doi.org/10.1016/S0953-7562(96)80102-X

Yao YJ, Spooner BM. 2003. The occurrence of *Geopora arenosa* in the British Isles. Kew Bulletin 58(1): 247–252. https://doi.org/10.2307/4119373

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Seychellomyces sinensis sp. nov. from China

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Abstract —An aquatic hyphomycete, *Seychellomyces sinensis*, isolated from China, is proposed as a new species. Phylogenetic analysis of combined ITS, LSU, 60S, and MCM7 sequences place *Seychellomyces* in *Ceratocystidaceae*. The new species, characterized by septate conidia and elliptical to cylindrical (sub)hyaline endoconidia, is distinguished from *S. hexagonus* by its larger, 1–3-septate conidia.

Key words—asexual fungi, *Microascales*, *Sordariomycetes*, taxonomy

Introduction

Aquatic hyphomycetes are fungi that most commonly occur on dead leaves in streams (Bärlocher 1992). These fungi are a polyphyletic group that play a key role in processing organic matter in freshwater (Belliveau & Bärlocher 2005). Traditionally, species have been identified through their conidial morphology, but phylogenetic placements of many species have not yet been determined. The introduction of molecular analysis to fungal taxonomy has recently verified the phylogenetic placement of some species (Duarte & al. 2015).

Seychellomyces Matsush. was proposed for a single species, *S. hexagonus* Matsush. (Matsushima 1981) and remains monotypic. It is characterized by

[§]These authors contributed equally to this research.

producing septate brown to pale brown conidia that are hexagonal in cross-section and aseptate hyaline endoconidia (Matsushima 1981, Seifert & al. 2011).

Several new hyphomycetes species have been reported from southwest China (Qiao & al. 2017a,b; 2018a,b, 2019; Yu & al. 2019). During further study of the aquatic hyphomycetes, a specimen with two types of conidia was found growing on submerged dicotyledonous leaves. The specimen was quite close to *Seychellomyces hexagonus* in its conidiogenous features but differed in possessing larger and more septate conidia. Here we describe it as a new species and present the phylogenetic position of *Seychellomyces* as determined by combined sequences of internal transcribed spacer (ITS), large subunit nuclear ribosomal RNA (LSU), 60S ribosomal protein gene RPL10 (60S), and the minichromosome maintenance complex component 7 (MCM7).

Materials & methods

Collection, fungal isolation, and morphological characterization

Submerged dicotyledonous leaves were collected from a stream in Sangsi, Guangxi Province, China. Samples were preserved in zip-lock plastic bags, labelled, and transported to the laboratory. Each rotted leaf was cut into several 3–4 × 4–5 cm fragments that were incubated on CMA (20 g cornmeal, 18 g agar, 40 mg streptomycin, 30 mg ampicillin, 1000 ml distilled water) for 5 days at room temperature. Individual conidia were isolated using a sterilized toothpick under a BX51 microscope and cultivated on CMA plates. Morphological observations were conducted on cultures growing on CMA, V-8 juice agar (200 ml Campbell V-8 juice, 3.0 g $CaCO_3$, 18 g agar, 800 ml distilled H_2O), and PDA (200 g potato, 20 g dextrose, 18 g agar, 1000 ml distilled H_2O) after incubation at 25°C for one week.

Pure cultures were deposited in the Herbarium of the Laboratory for Conservation and Utilization of Bio-resources, Yunnan University, Kunming, Yunnan, P.R. China (YMF; formerly Key Laboratory of Industrial Microbiology and Fermentation Technology of Yunnan).

DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from fresh mycelia grown on potato dextrose agar (PDA) at 25°C as described by Turner & al. (1997). Four gene regions were amplified: ITS with primer pair ITS5/ITS4 (White & al. 1990); LSU with LROR/LR7 (Vilgalys & Hester 1990); 60S ribosomal protein RPL10 (60S) with 60S-506F/60S-908R (Nel & al. 2018); and MCM7 with MCM7-for/MCM7-rev (Nel & al. 2018). The PCR thermal cycle programs for the amplifications of these four DNA fragments followed those described in de Beer & al. (2014). PCR products were then purified

Table 1. Strains and GenBank accession numbers of sequences used in molecular phylogenetic analyses

Species	Strain	GenBank number			
		ITS	LSU	60S	MCM7
Ambrosiella xylebori	CMW110.61	NR_144921	KM495318	KM495495	KM495407
Berkeleyomyces basicola	CMW6714	MF952423	MF948658	MF967072	MF967079
	CMW49351	MF952428	MF948659	MF967075	MF967102
	CMW25439	MF952427	—	—	MF967099
Berkeleyomyces rouxiae	CMW5472	MF952406	MF948657	MF967074	MF967080
	CMW14219	MF952402	MF948660	MF967076	MF967086
Ceratocystis diversiconidia	CMW22445	FJ151440	KM495334	KM495511	KM495423
Ceratocystis eucalypticola	CMW11537	FJ236723	KM495339	KM495516	KM495428
Ceratocystis fimbriata	CMW15049	AY953378	KM495343	KM495520	KM495432
Ceratocystis pirilliformis	CMW6579	AF427105	KM495365	KM495542	KM495453
Ceratocystis platani	CMW14802	DQ520630	KM495366	KM495543	KM495454
Chalaropsis ovoidea	CMW22733	FJ411343	KM495400	KM495577	KM495487
Chalaropsis thielavioides	CMW22736	FJ411342	KM495402	KM495579	KM495489
Davidsoniella australis	CMW2333	FJ411325	KM495396	KM495573	KM495483
Davidsoniella eucalypti	CMW3254	FJ411327	KM495338	KM495515	KM495427
Endoconidiophora coerulescens	CMW26365	FJ411322	KM495329	KM495506	KM495418
Endoconidiophora douglasii	CMW26367	NR_120295	KM495335	KM495512	KM495424
Graphium pseudormiticum	CMW503	AY148186	KM495390	KM495567	KM495477
Huntiella chinaeucensis	CMW24658	JQ862729	KM495327	KM495504	KM495416
Huntiella moniliformis	CMW10134	FJ151422	KM495355	KM495532	KM495443
Huntiella oblonga	CMW23803	EU245019	KM495359	KM495536	KM495447
Seychellomyces sinensis	YMF1.04178	KU549178	MG830704	MK554714	MK554715
Thielaviopsis cerberus	CMW36668	NR_145225	KM495326	KM495503	KM495415
Thielaviopsis musarum	CMW1546	JX518325	KM495357	KM495534	KM495445
Thielaviopsis paradoxa	CMW36689	JX518342	KM495363	KM495540	KM495451
Thielaviopsis radicicola	CMW1032	KF612023	KM495371	KM495548	KM495459

using a Bioteke DNA Gel/PCR Purification Kit and forward and reverse sequenced with a LI-COR 4000L automatic sequencer, using a Thermo Sequenase-kit as described by Kindermann & al. (1998).

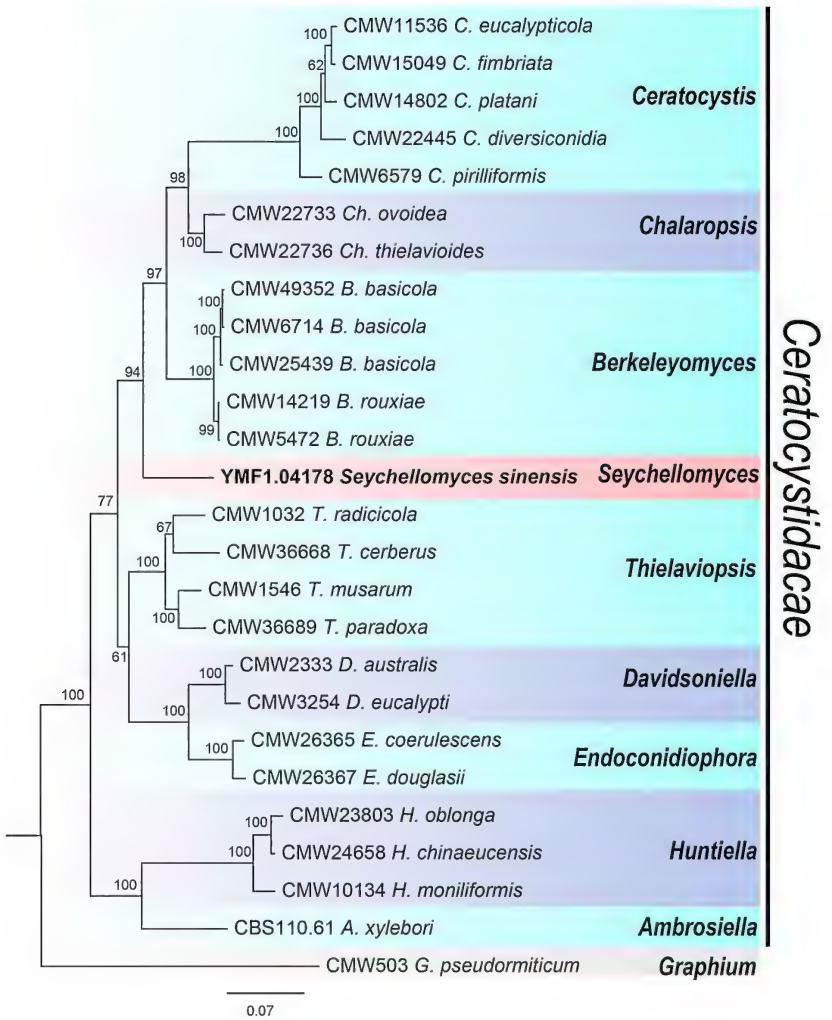

Plate 1. MrBayes tree generated from a combined alignment of ITS, LSU, 60S, and MCM7 sequences of *Ceratocystidaceae*, with *Graphium pseudormiticum* (*Microascaceae*) as outgroup.

Sequence alignment and phylogenetic analysis

The sequence dataset contained the ITS, LSU, 60S and MCM7 sequence data for 21 species representing eight genera in the *Ceratocystidaceae* (*Microascales*). *Graphium pseudormiticum* M. Mouton & M.J. Wingf. (*Microascaceae*) was chosen as outgroup based on Nel & al.'s (2018) phylogenetic study, and the sequence data were downloaded from the NCBI GenBank database (Table 1).

DNA sequence data were aligned using ClustalX 1.83 (Higgins 1994) with default parameters, and the consensus sequences were adjusted manually and linked in BioEdit v.7.0 (Hall 1999). MrBayes (Ronquist & Huelsenbeck 2003) was used to calculate the concatenated sequence-based tree, with following parameters: ngen = 1,000,000; samplefr = 1000; printfr = 1000.

Phylogenetic results

The final alignment included a total of 2022 base pairs. The phylogenetic tree was generated by combining the ITS, LSU, 60S, and MCM7 sequences and using Bayesian analysis. The topology of the tree is shown with the bootstrap support value for each node (Plate 1). The tree supports *Seychellomyces* within the *Ceratocystidaceae;* the genus does not group with any other genera but is associated with the lineage (94% support) containing *Berkeleyomyces*, *Chalaropsis*, and *Ceratocystis* in a basal position.

Taxonomy

Seychellomyces sinensis Z.F. Yu & Hua Zheng, Plate 2

MB 829084

Differs from *Seychellomyces hexagonus* by its larger 1–3-septate conidia.

Type: China, Guangxi province, Shangsi, Shiwan Mountain, 21°34′49″N 109°14′20″E, alt. 23 m, on decaying leaf of an unidentified broadleaf tree, 7 July 2013, Z.F. Yu. (**Holotype**, YMFT 1.04178 [permanent slide]; ex-type living culture, YMF 1.04178; GenBank KU549178, MG830704, MK554714, MK554715).

Etymology: From the Latin, *sinensis,* for the country of origin, China.

Colonies attaining 3 cm diam. after 7 days on CMA, effuse, pale brown to brown. Mycelium sparse, hyphae branched, septate, hyaline or pale brown. Conidiophores arising from the creeping hyphae, cylindrical, 2(–5)-septate, erect, unbranched, smooth, 12–40 × 5–7 µm (narrowing to 3.5–5 µm at the apex). Conidiogenous cells monoblastic, integrated, terminal, determinate. Conidia ellipsoidal, brown to pale brown, smooth or sometimes with a warty surface, growing singly or 2–3 in chains, 1–3-septate (mainly 2-septate), with short (4–13 µm long) cylindrical conidiophore remnants at both ends, in cross-section hexagonal (occasionally pentagonal or quadrangular). Endoconidia originating endogenously within phialides, catenulate, variable in length, hyaline or subhyaline, elliptical to cylindrical, truncate at ends, occasionally repetitiously germinating by producing a short conidiophore from which a phialide is formed to produce a chain of endoconidia. Chlamydospores ochraceous, globose, smooth, 5–10 µm in diam.

Conidial sizes on different media—On V-8 juice agar: 1-septate, 20–23 × 12–16 µm, total length (including short cylindrical conidiophore remnants) 34–39 µm; 2-septate, 29–39 × 16–21 µm, total length 40–56 µm; 3-septate 35–49 × 11–15 µm, total length 55–35 µm; endoconidia 5–21 × 3–5.7 µm.

On PDA: 1-septate, 20–27 × 13–21 µm, total length 31–45 µm; 2-septate, 38–50 × 19–30 µm, total length 42–69 µm; 3-septate 38–56 × 19–27 µm, total length 53–73 µm; endoconidia 7–39 × 3–5 µm.

On CMA: 1-septate, 23–30 × 15–21 µm, total length 34–47 µm; 2-septate, 31–51 × 19–31 µm, total length 42–70 µm; 3-septate, 42 × 20 µm, total length 58 µm; endoconidia 6–42 × 3–5 µm.

Discussion

Seychellomyces has been treated as monotypic since the genus was established in 1981. Based on characteristic of conidia and endoconidia, our strain was easily identified to *Seychellomyces*. However, *S. sinensis* is distinguished from the type species, *S. hexagonus*, by having 1–3-septate conidia, while conidia of *S. hexagonus* are always 2-septate. In addition, conidia of *S. hexagonus* are always hexagonal in cross-section (Matsushima 1981), whereas those of *S. sinensis* are mostly hexagonal, but occasionally pentagonal or quadrangular. The size of conidia of *S. sinensis* differs based on growth medium and conidial septation; but on V-8 juice agar medium the 2-septate conidia of *S. sinensis* are larger than those of *S. hexagonus* (25–33 × 14–18 µm; Matsushima 1981). Globose chlamydospores were observed in culture of *S. sinensis*, but not mentioned in *S. hexagonus*.

Among *Ceratocystidaceae*, only *Thielaviopsis* sensu lato has a superficial resemblance to *Seychellomyces* in conidial shape (Seifert & al. 2011). However, *Thielaviopsis*, *Chalaropsis*, and *Ceratocystis* significantly differ from *S. sinensis* phylogenetically.

Acknowledgments

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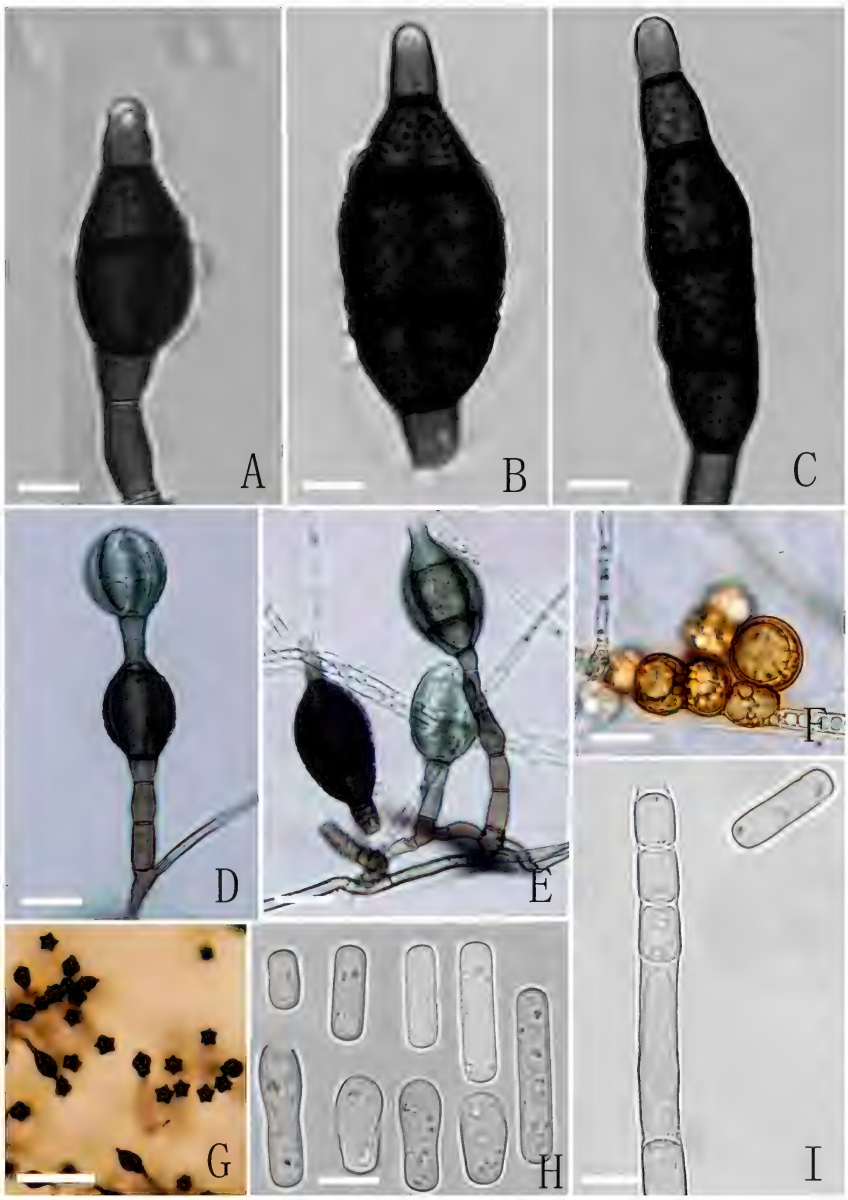

Plate 2. *Seychellomyces sinensis* (holotype, YMF 1.04178): A–C. Conidia; D, E. Conidia with conidiophores; F. Chlamydospores; G. Cross-section of conidia. H. Endoconidia. I. Conidiogenous cells of endoconidia. Scale bars: A–C, F, H, I = 10 μm; D, E = 20 μm; G = 100 μm.

Literature cited

Bärlocher F. 1992. The ecology of aquatic hyphomycetes. Springer Berlin Heidelberg.

Belliveau MJ, Bärlocher F. 2005. Molecular evidence confirms multiple origin of aquatic hyphomycetes. Mycological Research 109: 1407–1417. https://doi.org/10.1017/s0953756205004119

de Beer ZW, Duong TA, Barnes I, Wingfield BD, Wingfield MJ. 2014. Redefining *Ceratocystis* and allied genera. Studies in Mycology 79:187–219. https://doi.org/10.1016/j.simyco.2014.10.001

Duarte S, Batista D, Bärlocher F, Cássio F, Pascoal C. 2015. Some new DNA barcodes of aquatic hyphomycete species. Mycoscience 56: 102–108. https://doi.org/10.1016/j.myc.2014.04.002

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98. https://doi.org/10.1007/978-1-4757-0905-6_31

Higgins DG. 1994. CLUSTAL V: multiple alignment of DNA and protein sequences. Methods in Molecular Biology 25: 307–318. https://doi.org/10.1385/0-89603-276-0:307

Kindermann J, El-Ayouti Y, Samuels GJ, Kubicek CP. 1998. Phylogeny of the genus *Trichoderma* based on sequence analysis of the internal transcribed spacer region 1 of the rDNA clade. Fungal Genetics and Biology 24: 298–309. https://doi.org/10.1006/fgbi.1998.1049

Matsushima T. 1981. Matsushima mycological memoirs no. 2. Matsushima Fungus Collection. Kobe. 68 p.

Nel WJ, Duong TA, Wingfield BD, Wingfield MJ, de Beer ZW. 2018. A new genus and species for the globally important, multihost root pathogen *Thielaviopsis basicola*. Plant Pathology 67: 871–882. https://doi.org/10.1111/ppa.12803

Qiao M, Huang Y, Deng C, Yu ZF. 2017a. *Tripospermum sinense* sp. nov. from China. Mycotaxon 132: 513–517. https://doi.org/10.5248/132.513

Qiao M, Du X, Bian ZH, Peng J, Yu ZF. 2017b. *Ellisembia pseudokaradkensis* sp. nov. from Hainan, China. Mycotaxon 132: 813–817. https://doi.org/10.5248/132.813

Qiao M, Guo JS, Tian WG, Yu ZF. 2018a. *Ellisembia hainanensis* sp. nov. from Hainan, China. Mycotaxon 133: 97–101. https://doi.org/10.5248/133.97

Qiao M, Li WJ, Huang Y, Xu JP, Zhang L, Yu ZF. 2018b. *Classicula sinensis*, a new species of basidiomycetous aquatic hyphomycetes from southwest China. MycoKeys 40: 1–12. https://doi.org/10.3897/mycokeys.40.23828

Qiao M, Tian WG, Castañeda-Ruiz RF, Xu JP, Yu ZF. 2019. Two new species of *Verruconis* from Hainan, China. MycoKeys 48: 41–53. https://doi.org/10.3897/mycokeys.48.32147

Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Seifert K, Morgan-Jones G, Gams W, Kendrick B. 2011. The genera of hyphomycetes. CBS Biodiversity Series 9. 997 p.

Turner D, Kovacs W, Kuhls K, Lieckfeldt E, Peter B, Arisan-Atac I, Strauss J, Samuels GJ, Börner T, Kubicek CP. 1997. Biogeography and phenotypic variation in *Trichoderma* sect. *Longibrachiatum* and associated *Hypocrea* species. Mycological Research 101: 449–459. https://doi.org/10.1017/s0953756296002845

Vilgalys R, Hester M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology 172: 4238–4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. 315–322, in: MA Innis & al. (eds). PCR Protocols: a guide to methods and applications. San Diego, CA. Academic Press. https://doi.org/10.1016/b978-0-12-372180-8.50042-1

Yu ZF, Lv YF, Feng B, Qiao M. 2019. *Lemonniera yulongensis* sp. nov. from Yunnan, China. Mycotaxon. 134:177–181. https://doi.org/10.5248/134.177

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Ramaria flavescentoides sp. nov. with clamped basidia from Pakistan

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Abstract—During an investigation of macrofungi from Pakistan, a new species with clamped basidia within *Ramaria* subg. *Laeticolora* and associated with *Abies pindrow* was found, which is proposed here as *Ramaria flavescentoides*. The new fungus morphologically resembles *R. flavescens* and clusters phylogenetically within the same clade. *Ramaria flavescentoides* is characterized by an obovate to obconic basidioma with pale orange to light orange branches and apices that are tinged light greenish grey, a monomitic hyphal system, ampulliform clamp connections, stellate crystals distributed within the upper branches, multiguttulate rhizomorphic strands, and oblong to ellipsoid verrucose basidiospores.

Key words—Himalayan moist temperate forest, ITS region, molecular identification, polychotomous branching

Introduction

Ramaria Fr. ex Bonord. (*Ramariaceae*) is a widespread non-gilled basidiomycete genus comprising nearly 220 species worldwide (Kirk & al. 2008). The basidiomata, which generally repeatedly branch dichotomously, are usually divided into stipe, branches, and apices. Important diagnostic characters include color, stipe consistency (fibrous vs gelatinous), odor, staining reactions to reagents, presence or absence of clamp connections, and spore size and ornamentation (Exeter & al. 2006). Villegas & al. (1999)

placed *Ramariaceae* in *Gomphales*, a placement also supported by molecular phylogenetic studies (Hibbett & al. 1997, Bruns & al. 1998, Humpert & al. 2001, Giachini 2004). *Ramaria,* which includes both saprobic and ectomycorrhizal species, is currently divided into four subgenera: *R.* subg. *Echinoramaria* Corner, *R.* subg. *Lentoramaria* Corner, *R.* subg. *Ramaria,* and *R.* subg. *Laeticolora* Marr & D.E. Stuntz (Marr & Stuntz 1973, Exeter & al. 2006). Previously these divisions were based on spore ornamentation, monomitic vs. dimitic rhizomorphic strands, humicolous vs. lignicolous substrate, and presence or absence of clamp connections.

Ramaria flavescentoides is described here based on morphological and molecular data as a new species in *R.* subg. *Laeticolora*. Both *R.* subg. *Laeticolora* and *R.* subg. *Ramaria* tend to develop larger fruiting bodies than *R.* subg. *Echinoramaria* and *R.* subg. *Lentoramaria*. *Ramaria* subg. *Laeticolora* is further characterized by non-striate basidiospores that are almost smooth to distinctly ornamented at 1000× (vs. striate in *R.* subg. *Ramaria*), tissues that are primarily non-amyloid (vs. amyloid in *R.* subg. *Ramaria*), single to fasciculate stipe bases, usually brightly colored branches and apices, and a terrestrial habit (Exeter & al. 2006).

Materials and methods

Collections

The sampling site was selected based on the richness and abundance of aphyllophoralean fungi in an area of probable high basidiomycete biodiversity in a moist temperate coniferous forest in Khyber Pakhtunkhwa, Pakistan where air temperatures range from –4 to 25°C, and the soil is loamy with gravel and variably sized stones. Field notes and photographs were made of all collections, and the specimen was morphologically characterized following Corner (1950). Features (stipe, branches, rhizomorphic hyphae) were measured microscopically at 1000× using a compound microscope equipped with an ocular micrometer. Measurements of basidiospores, basidia, cystidia, and rhizomorphic hyphae were determined from at least 20 measurements per character. Small (~1 cm) portions of the hymenium were stored in 2% CTAB buffer in 1.5 ml Eppendorf vials and stored at –20°C for molecular analysis; the remaining material was dried using a fan heater and stored in sealed plastic bags. The collections are curated at the mycological herbarium at the University of the Punjab, Lahore, Pakistan (LAHMH).

DNA extraction, PCR amplification & sequencing

Hymenial tissue was removed with sterile forceps for DNA extraction by a CTAB method following Gardes & Bruns (1993), modified for Gene-Clean silica emulsion binding and purification. The polymerase chain reaction (PCR) followed Gardes &

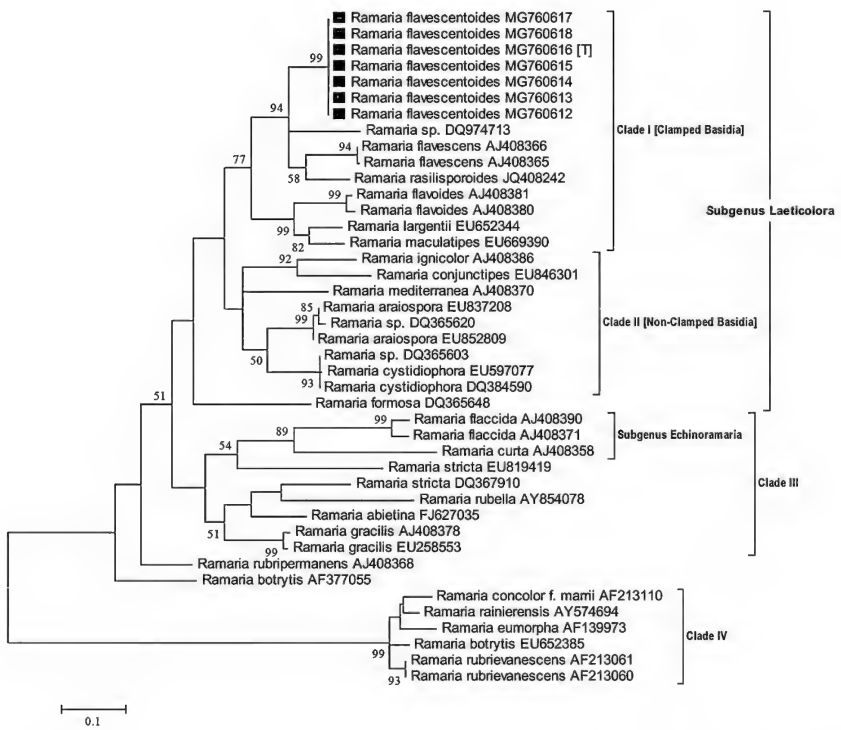

Fig. 1. Maximum Likelihood phylotree of the evolutionary history of ITS sequences of *Ramaria* species, including *R. flavescentoides*. The tree with the highest log likelihood (–10063.0715) is shown, drawn to scale with branch lengths indicating the number of substitutions per site. Bootstrap support is shown next to the branches.

Bruns (1993), using the fungus-specific ITS1F primer (CTTGGTCATTTAGAGGAAGT) and the eukaryotic ITS4 primer (TCCTCCGCTTATTGATATGC) to amplify the nuclear rDNA-ITS region. The hot-start enzyme JumpStart catalyzed the PCR with 2 min at 94°C; followed by 30 cycles of 30 s at 94°C, 30 s at 53°C, and 40 s + 5 s per cycle at 72°C; and finishing with 5 min at 72°C. The PCR products were purified with QIAquick, sequenced bi-directionally using the reverse and forward primers and BigDye 3.1 on an ABI 3730 DNA sequencer, and edited in Sequencher 4.5 by Jodrell Laboratory, Royal Botanical Gardens, Kew, UK. The DNA sequences were BLAST searched at NCBI (https://www.ncbi.nlm.nih.gov/) and submitted to GenBank. Sequences were aligned and corrected manually for phylogenetic analysis with MacClade 4.08. Percentage identities were calculated using DNAStar software.

Phylogenetic analysis

The rDNA ITS sequences were aligned with sequences of other *Ramaria* species selected from GenBank. The original dataset comprising 1108 characters including gaps was trimmed by 252 characters excluded from 3′ and 5′ ends of the sequences. All gaps were treated as missing data. Of the 856 characters (of 'unord' type and equally weighted) analysed, 202 characters were constant, 616 were parsimony-uninformative, and 507 were parsimony-informative. The Branch-and-Bound search method was used for bootstrapping with 1000 bootstrap replicates in MEGA5 (Tamura & al. 2011).

Results

ITS rDNA-based identification and phylogenetic analysis

The amplification of the rDNA ITS from *Ramaria flavescentoides* produced 630–665 bp fragments. The consensus sequence of *R. flavescentoides* showed 91% identity and 65% query coverage with an undescribed *Ramaria* sp. (DQ974713.1) and 88% identity and 75% query coverage with *R. flavescens* (AJ408365).

Species included in this study were selected from all four *Ramaria* subgenera based on the diagnostic characters of each subgenus. The maximum likelihood phylogram represents four clades (Fig. 1). Clade I represents species belonging to *R.* subg. *Laeticolora* with clamped basidia, Clade II represents species belonging to *R.* subg. *Laeticolora* with non-clamped basidia, and Clades III and IV represent species belonging to *R.* subg. *Echinoramaria*, *R.* subg. *Lentoramaria,* and *R.* subg. *Ramaria*. The Clade I sequences from *R. flavescentoides* clustered with those from *R. flavescens*, *R. flavoides*, *R. largentii*, *R. maculatipes*, *R. rasilisporoides*, and *Ramaria* sp. (bootstrap = 77%). Within the clade, *R. flavescentoides* was sister to *R. flavescens*, *R. rasilisporoides*, and *Ramaria* sp. (bootstrap = 94%). Percentages of genetic characters shared by *R. flavescentoides* with other species were: *Ramaria* sp.—83%, *R. flavescens*—82%, *R. largentii*—81%, *R. rasilisporoides*—79%, *R. maculatipes*—78%, and *R. flavoides*—77%.

Taxonomy

Ramaria flavescentoides Hanif & Khalid, **sp. nov.** Fig. 2

MB 823965

Differs from *Ramaria flavescens* by its obconic to obovate basidioma, its non-bulbous fasciculate stipe, and the presence of stellate crystals in the upper branches.

Type: Pakistan. Khyber Pakhtunkhwa province, Hazara Division, Ayubia–Khanspur, 34°01′24″N 73°24′14″E, 2159 m asl, under *Abies pindrow*, 8 August 2009, M. Hanif MH88 (**Holotype**, LAHMH0889; GenBank MG760616).

Etymology—Named for its macroscopic resemblance with *R. flavescens*.

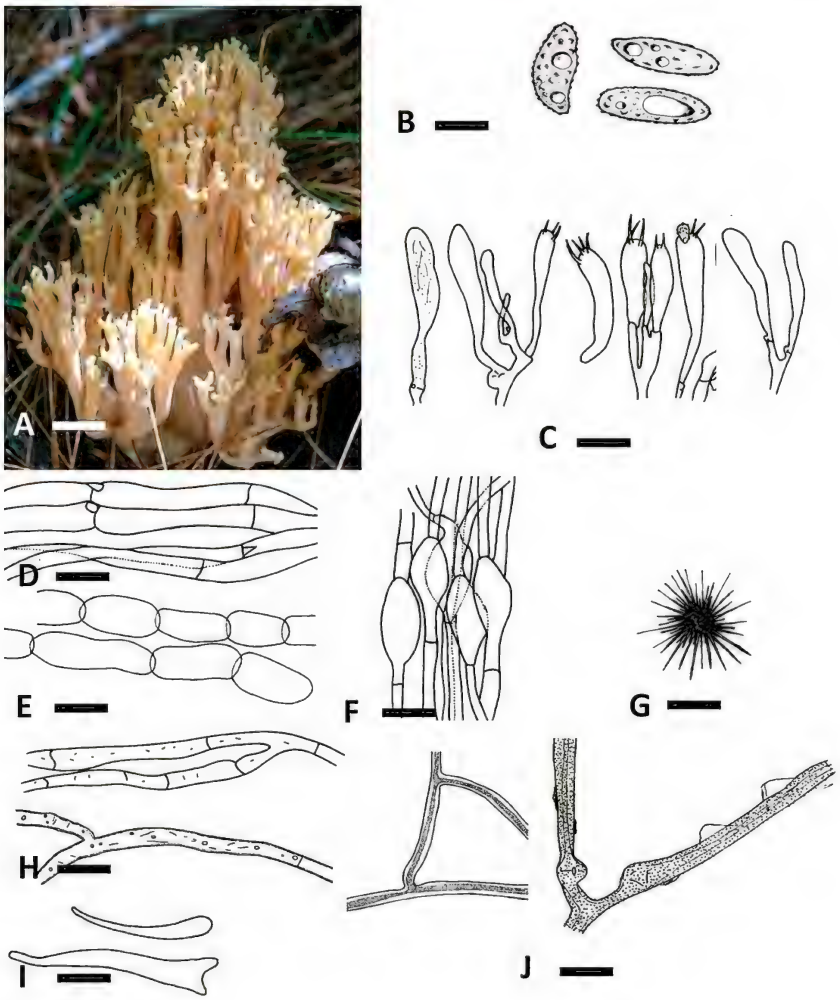

Fig. 2. *Ramaria flavescentoides* (holotype, LAHMH0889). A. Basidiocarps; B. Basidiospores; C. Basidia and cystidia; D. Stipe tramal hyphae; E. Overlapped cellular hyphae of upper branches; F. Ampulliform tramal hyphae of upper branches; G. Stellate crystals among stipe hyphal mass; H. Cellular hyphae of upper branches; I. Tramal elements of upper branches; J. Dimitic hyphae of rhizomorph. Scale bars: A = 2 cm; B, C = 3 µm; D, G = 15 µm; E, H = 17 µm; F = 13.5 µm; I = 6 µm; J = 37.5 µm.

Basidioma 13–15 cm high, ≤10 cm broad, repeatedly branched, usually obovate to obconic in outline; orange to light orange on drying. Stipe ≤3(–

4) cm high, compound to fasciculate in groups of 2–5, orange white at the base, upward pale orange, tapering gradually downward into a basal mycelial mat or white tomentum covering the stipe base. Rhizomorphs white, involving significant amounts of substrata, drying light orange. Flesh white, solid, gelatinous or slippery, dried flesh firm but easily penetrated. Major branches 4–6, 1.5–2 cm thick, stout, more or less terete, ascending, orange white below, upward pale. Branches 4–7 ranks, ascending, polychotomous, smooth and gelatinous from exterior, pale orange; axils more or less rounded. Apices 2–4 mm long, acute to blunt, light orange up to the tips with light greenish grey tint. Taste, odor, and macrochemical reactions not recorded.

Stipe tramal hyphae 6–11 µm wide, hyaline, thin-walled, parallel, slightly ampulliform, occasionally clamped, grainy encrusted with crystalline material of various shapes; gloeoplerous hyphae not observed. Tramal hyphae of upper branches 6–8 µm diam., hyaline, thin-walled, parallel, ampulliform; skeletal generative hyphae present, clamps occasional, stellate crystalline material 32 µm diam. Hyphae of basal mat monomitic, 3–4 µm diam., thin-walled, branched, clamped, 6 µm thick at clamps, grainy encrusted with crystalline material of various shapes; generative hyphae 7–10 µm diam. Rhizomorphic hyphae smooth to encrusted, diameters vary. Subhymenium rudimentary, hyphal structure is monomitic with thin-walled and thick-walled hyphae of various diameters, overlapped, septate; frequent ampulliform hyphae (27 × 15 µm). Hymenium thick during maturation. Basidia 32–50(–70) × 9–12 µm, clavate, clamped; contents homogeneous when young, slightly grainy, hyaline or slightly yellowish, slightly cyanophilous, 4-spored; sterigmata 6–8 µm high. Basidiospores (10.7–)11–13(–15) × (4.8–)5–6(–7) µm, verrucose, multiguttulate when mature, oblong to ellipsoid, slightly curved at one end, contents weakly cyanophilous; wall moderately thick; ornamentation strongly cyanophilous, roughened outline of low coarse warts and delicate meandering ridges covering significant areas of wall.

Habitat & distribution—Terrestrial, solitary, under *Abies pindrow*. Known only from the type area, Ayubia–Khanspur, fruiting in July–August at 1900–2200 m asl.

Additional specimens examined—**PAKISTAN. Khyber Pakhtunkhwa province, Hazara Division**, Ayubia–Khanspur, 34°01′24″N 73°24′14″E, 2159 m asl, 17 Aug 2009, M. Hanif MH136 (LAHMH1789.1; GenBank MG760618); Helipad, 34°01′31″N 73°25′19″E, 1974 m asl, 25 Jul 2008, M. Hanif MH253 (LAHMH2578.2; GenBank MG760612); M. Hanif MH254 (LAHMH2578.3; GenBank MG760617);

M. Hanif MH96 (LAHMH2578.5; GenBank MG760615); M. Hanif MH101 (LAHMH2578.1; GenBank MG760613); 16 Aug 2009, M. Hanif MH97 (LAHMH1689; GenBank MG760614).

Comments—*Ramaria flavescentoides* is characterized by medium sized obovate to obconic basidiomata with pale orange to light orange colored branches and lightly tinged greenish grey apices and a compound to fasciculate stipe that narrows gradually downward into a mat of white mycelia and rhizomorphic strands.

The new species resembles *R. flavescens* (Schaeff.) R.H. Petersen in shape, branching pattern, and basidioma color, also sharing a similar spore shape and ornamentation and the presence of basidial clamp connections. However, our specimens differ morphologically from *R. flavescens,* which produces smaller (10 × 7 cm) obpyriform profusely branched basidiomata with a rather abrupt ball surmounting the stipe and smaller basidiospores (11–12.5 × 4.8–5.6 µm; Petersen 1974). The presence of stellate crystalline material in the hyphae of upper branches is another important character of *R. flavescentoides*. Similar crystals have been reported by Zhang & al (2005) in the stipe and upper branches of *Ramaria luteoaeruginea* P. Zhang & Zhu L. Yang in *R.* subg. *Echinoramaria*. Phylogenetically, *R. flavescentoides* nests adjacent to *R. flavescens*; and *R. flavescentoides* and *R. flavescens* cluster together in clade I with other species having clamped basidia (Fig. 1).

Ramaria flavescentoides shares similar branching pattern, clamped basidia, warted (verrucose) spores with morphological similarities with its phylogenetic sister species, *R. rasilisporoides* Exeter, which clusters in the same clade with strong (94%) bootstrap support (Fig. 1). *Ramaria rasilisporoides* differs from *R. flavescentoides* in its pale yellow basidiomata, amyloid stipe tissues, deeply rooting stipe with numerous abortive branchlets, and smaller basidiospores (Exeter & al. 2006).

Ramaria mairei Donk, the only species of subg. *Laeticolora* previously reported from Pakistan (Ahmad 1972; Ahmad & al. 1997), is distinguished by larger (18 cm) basidiomata, a 8 cm high stipe, numerous pale or deep lemon to pale ochraceous and rather lax or fairly crowded branches, and slightly longer (10–15-µm) oblong to ellipsoid roughened pale ochraceous basidiospores (Ahmad 1972).

Phylogenetic analysis also supports the morphological identification. *Ramaria flavescentoides* clusters with the clamped species of subg. *Laeticolora*. This clade seems to be paraphyletic and originated with non-clamped species of this subgenus with unsupported bootstrap frequency.

Humpert & al. (2001) reported the genus *Ramaria* as polyphyletic, which our analyses support.

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Literature cited

Ahmad S. 1972. Basidiomycetes of Pakistan. Biological Society of Pakistan. Monograph 6, Lahore.

Ahmad S, Iqbal SH, Khalid AN. 1997. Fungi of Pakistan. Sultan Ahmad Mycological Society of Pakistan, Department of Botany, University of Punjab, Quaid-e-Azam Campus, Lahore.

Bruns TD, Szaro TM, Gardes M, Cullings KW, Pan JJ, Taylor DL, Horton TR, Kretzer A, Garbelotto M, Li Y. 1998. A sequence database for the identification of ectomycorrhizal basidiomycetes by phylogenetic analysis. Molecular Ecology 7: 257-272. https://doi.org/10.1046/j.1365-294X.1998.00337.x

Corner EJH. 1950. A monograph of *Clavaria* and allied genera. Annals of Botany Memoirs. 1. 740 p.

Exeter RL, Norvell L, Cázares E. 2006. *Ramaria* of the Pacific Northwestern United States. United States Department of Interior, Bureau of Land Management: Salem, Oregon USA. 156 p.

Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for basidiomycetes application to the identification of mycorrhizae and rusts. Molecular Ecology 2: 113–118 https://doi.org/10.1111/j.1365-294X.1993.tb00005.x

Giachini AJ. 2004. Systematics of the *Gomphales:* the genus *Gomphus* Pers. sensu lato. PhD Dissertation, Oregon State University, Corvallis, OR, USA

Hibbett DS, Pine EM, Langer E, Langer G, Donoghue MJ. 1997. Evolution of gilled mushrooms and puffballs inferred from ribosomal DNA sequences. Proceedings of the National Academy of Sciences of the United States of America 94: 12002–12006. https://doi.org/10.1073/pnas.94.22.12002

Humpert AJ, Muench EL, Giachini AJ, Castellano MA, Spatafora JW. 2001. Molecular phylogenetics of *Ramaria* and related genera: evidence from nuclear large subunit and mitochondrial small subunit rDNA sequences. Mycologia 93: 465–477. https://doi.org/10.2307/3761733

Kirk PM, Cannon PF, Minter DW, Stalpers JA. 2008. Ainsworth & Bisby's dictionary of the fungi. 10th edn. CAB International, U.K. https://doi.org/10.1079/9780851998268.0000

Marr CD, Stuntz DE. 1973. *Ramaria* of western Washington. Bibliotheca Mycologia 38. 232 p.

Petersen RH. 1974. Contribution toward a monograph of *Ramaria*. I. Some classic species redescribed. American Journal of Botany 61: 739–748. https://doi.org/10.1002/j.1537-2197.1974.tb12296.x

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution 28(10): 2731–2739. https://doi.org/10.1093/molbev/msr121

Villegas M, de Luna E, Cifuentes J, Torres AE. 1999. Phylogenetic studies in *Gomphaceae* sensu lato (*Basidiomycetes*). Mycotaxon 70: 127–147.

Zhang P, Yang ZL, Ge ZW. 2005. Two new species of *Ramaria* from southwestern China. Mycotaxon 94: 235–240.

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Regional annotated mycobiota new to the Mycotaxon website

Abstract—In May, Mycotaxon added the following new annotated species distribution list to our 132 previously posted free-access fungae. The 24-page "Aphyllophoroid fungi in Teide National Park (Tenerife, Canary Islands)" by Esperanza Beltrán-Tejera, Jesús Laura Rodríguez-Arma, Miguel Jonathan Díaz Armas, and Luis Quijada may be downloaded from our website via http://www.mycotaxon.com/mycobiota/index.html

EUROPE

Portugal & Spain (Iberian Peninsula)

Esperanza Beltrán-Tejera, Jesús Laura Rodríguez-Arma, Miguel Jonathan Díaz Armas, Luis Quijada. Aphyllophoroid fungi in Teide National Park (Tenerife, Canary Islands). 24 p.

Abstract—Data on aphyllophoroid fungi in Teide National Park Tenerife are summarized, and 102 species are recorded. Twenty eight species are new to Tenerife, out of which 17 are also new records for the Canary Islands (*Athelia pyriformis, Cartilosoma ramentaceum, Ceriporia excelsa, Dendrocorticium lilacinoroseum, Hyphoderma sibiricum, Hyphodermella rosae, Hyphodontiella multiseptata, Melzericium bourdotii, Phanerochaete avellanea, Phanerochaete cremeo-ochracea, Phlebia griseoflavescens, Phlebia lacteola, Phlebia lilascens, Phlebia tuberculata, Sistotrema pistilliferum, Skeletocutis amorpha,* and *Tubulicrinis angustus*). The list is supplemented with 7 species of *Peniophora* recorded for this area, published previously (Díaz Armas et al. 2019).

Mycobiota of the two highest mountain areas of the Canary Islands (Teide National Park in Tenerife and Taburiente National Park in La Palma) are compared regarding their richness of genera, species, abundance and substrates. Aphyllophoroid fungi were found on 16 endemic vascular species, the majority were on *Spartocytisus supranubius,* which is dominant in both abundance and distribution in the study area.

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Book reviews and notices*

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Abstract—Books reviewed include Syllabus of Plant Families—A. Engler's Syllabus der Pflanzenfamilien. 1/3 *Basidiomycota* and *Entorrhizomycota* by Begerow, McTaggart, and Agerer (W. Lang ed.) and Fungi lepiotoidei (*Agaricaceae*) edited by Zhu-Liang Yang (2019).

General

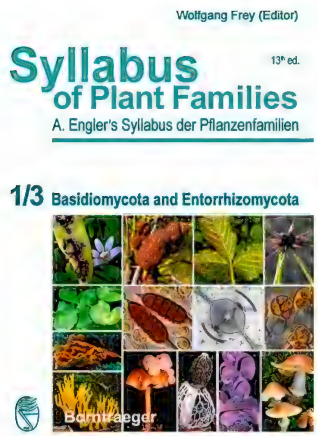
Syllabus of Plant Families—A. Engler's Syllabus der Pflanzenfamilien. 1/3 *Basidiomycota* and *Entorrhizomycota*. 13th ed. By Wolfgang Frey (ed.) 2018. Borntraeger Science Publishers, Johannesstr. 3A, 70176, Stuttgart, Germany. xii+471p. 43 figs. ISBN 978-3-443-01098-0. Price (excl. postage): € 139. www.borntraeger-cramer.com/9783443010980

Two years after the volume on *Ascomycota* (Jaklitsch & al. 2016), the current work was published, giving an overview of the *Basidiomycota* and the *Entorrhizomycota*.

Only three authors wrote the text: Dominik Begerow and Alistair McTaggart took care of the *Pucciniomycotina*, *Ustilagomycotina*, and *Entorrhizomycota*, whereas Reinhard Agerer is the author for the *Agaricomycotina*.

This is a systematic overview, incorporating the newest insights and giving standardized descriptions for all genera in these groups. As such it replaces long out-of-date works such as Singer's 1986 monumental "The

* Book reviews or books for consideration for coverage in this column should be sent to the Editor-in-Chief <editor@mycotaxon.com> 6720 NW Skyline, Portland OR 97229 USA.



Agaricales in modern taxonomy," although the present work lacks keys and infrageneric classifications and is much wider in scope.

I am impressed with the morphological descriptions provided, which present a wealth of knowledge.

It is always a bit of a balancing act which genera to include, as modern data are not available for all genera. There are names that have not been used in decades, but this does not mean that they do not represent good solid genera. In some genera, most species—but not all—have been transferred to a more fitting genus, such as is the case for *Torrendia*. But as one Australian *Torrendia* species has not yet been combined in *Amanita*, the genus is still treated here separately.

Unfortunately, there are quite a number of strange mistakes, such as the statement that *Saproamanita* species are ectomycorrhizal. This genus was explicitly erected to accommodate the non-ectomycorrhizal species in *Amanita*, as is also reflected in the genus name (Redhead & al. 2016). Another example is *Notholepiota,* which here is still included in the *Agaricaceae*, although it was shown to be based on a secotioid bolete species, now called *Boletus semigastroideus* (Nuhn & al. 2013). Some genera, such as *Bonomyces* and *Cruentomycena* are not included, for unknown reasons.

There are also differences between the treatments of the *Pucciniomycotina* and *Ustilagomycotina* on the one hand, and the *Agaricomycotina* on the other. For instance, in *Agaricomycotina* the type species of a genus is only given for monotypic genera but is always cited for genera in the other two subphyla. Discussion on genus borders etc. are also given for the genera in the first two subphyla, and absent in the *Agaricomycotina*.

Of course, the description of new families and genera did not grind to a halt when this work was sent to the publisher. *Mythicomycetaceae* (Vizzini et al. 2019) and *Sarcoporiaceae* (Audet 2018) have recently been distinguished at the family level, and *Zhulangiomyces* (Redhead 2019) and *Clitopaxillus* (Alvarado & al. 2018) are just two examples of recently published genera in the *Agaricales*.

This overview is an especially useful and thorough piece of work that hopefully will be the standard for years to come. As such the SYLLABUS 1/3 deserves a place on many mycologists' bookshelves.

Alvarado P, Moreau P-A, Dima B, Vizzini A, Consiglio G, Moreno G, Setti L, Kekki T, Huhtinen S., Liimatainen K, Niskanen T. 2018. *Pseudoclitocybaceae* fam. nov. (*Agaricales*, *Tricholomatineae*), a new arrangement at family, genus and species level. Fungal Diversity 90: 109–133. https://doi.org/10.1007/s13225-018-0400-1

Audet S, 2018. *Sarcoporiaceae*. Mushrooms nomenclatural novelties no. 18: 1–6.

Jaklitsch W, Baral H.-O, Lücking R, Lumbsch HT, Frey W (eds). 2016. Syllabus of Plant Families. A. Engler's Syllabus der Pflanzenfamilien Part 1/2: *Ascomycota*.

Nuhn ME, Binder M, Taylor AFS, Halling RE, Hibbett DS, 2013. Phylogenetic overview of the *Boletineae*. Fungal Biology 117: 479–511. https://doi.org/10.1016/j.funbio

Redhead SA, 2019. Nomenclatural novelties. Index Fungorum 385: 1.

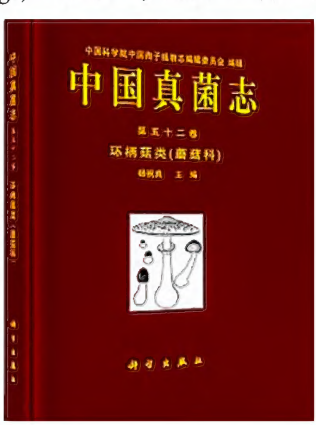
Redhead SA, Vizzini A, Drehmel DC, Contu M. 2016. *Saproamanita,*a new name for both *Lepidella* E.-J. Gilbert and *Aspidella* E.-J. Gilbert (*Amaniteae*, *Amanitaceae*). IMA Fungus 7: 119–129. https://doi.org/10.5598/imafungus.2016.07.01.07

Singer, R. The *Agaricales* in modern taxonomy. Ed. 4. Koeltz Scientific Books, Koenigstein, Federal Republic of Germany, 908 p., 88 pl.

Vizzini A., Consiglio G., Marchetti M., 2019. *Mythicomycetaceae* fam. nov. (*Agaricineae*, *Agaricales*) for accommodating the genera *Mythicomyces* and *Stagnicola*, and *Simocybe parvispora* reconsidered. FUSE 3: 41–56. https://doi.org/10.3114/fuse.2019.03.05

BASIDIOMYCETES

Fungi lepiotoidei (*Agaricaceae*). By Zhu-Liang Yang (ed.). 2019. FLORA FUNGORUM SINICORUM VOL. 52. http://www.sciencep.com Science Press, Beijing, China. 228 p. 2 colour plates, 112 black & white figures, Hardcover. ISBN 978-7-03-060680-8. Price (excl. postage): 198.00 ¥; cited at $76 by www.chinascientificbooks.com



This 52nd volume in the series that aims at providing keys and descriptions of all fungi in China focuses on the lepiotoid fungi, to be more precise, all agaricoid genera in the *Agaricaceae* except *Agaricus* and *Micropsalliota*, a total of 14 genera. Most of these are white-spored, but some species in *Chlorophyllum* and the genera *Heinemannomyces*, *Hymenagaricus*, and *Melanophyllum* have coloured basidiospores.

An introduction to the group gives an overview of the recent literature, classification and phylogeny of the group (which represents only part of a monophyletic clade), and the morphology.

After the key to the genera, the genera are treated in alphabetical order, with keys to species, descriptions, and high-quality line drawings of all species that have been confirmed for the country. All collections on which the descriptions are based are listed. Those species listed in earlier works whose presence is questionable are briefly discussed. For 15 species, colour photos are provided. An extensive list of references and an index complete the work.

This book is the result of 15 years of work by Prof. Zhu-Liang Yang and his students (now professors themselves) Zai-Wei Ge and Jun-Feng Liang.

Lepiota has the highest number of taxa (48)—it interesting is to see how some species have a distribution area spanning from western Europe throughout the Northern Hemisphere into China, whereas others, especially those from southern, tropical China, have a much more limited distribution. Thanks to DNA comparisons of collections from Europe, North America and China, real distribution patterns emerge. In the past, when neither these techniques nor literature were available, names were adopted indiscriminately from other regions.

Some species listed under *Lepiota* (*L. shixingensis* and *L. furfuraceipes*) belong to *Leucoagaricus*. *Hymenagaricus taiwanensis* is now considered in the genus *Xanthagaricus*.

The last word on the Chinese lepiotoids has not been said yet—I am convinced that there are more species in *Leucoagaricus* than presented here, but that does not diminish the value of the present work, a value that extends far beyond China. The only drawback, of course, is the language, as the book is written in Chinese, but fortunately the names are presented in Latin.

Beltraniopsis cyclobalanopsidis sp. nov.
(Xiao & al.— Fig. 1, p. 303)